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METHODS

PART 3

BIOLOGICAL CHEMISTRY

DATE: 12.OCT.81

250109187

Source: https://www.industrydocuments.ucsf.edu/docs/lghl0000

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Determination of acid phosphatase (EC 3.1.3.2) activity

Principle:	fluorometric determination of 4-methylumbelliferone at alkaline pH after hydrolysis of 4-methyl-umbelliferyl phosphate at acid pH
Time	
Sampling:	on day ...
Determination:	on day of sampling
Sample material and quantity:	fractionated pulmonary macrophages, (1) 1o ul (2) 2o ul
Results expressed in:	(1) mU/fraction (2) U/1oE6 macrophages (3) U/1oE6 cells (a)
Equipment:	thermostat: type "Eppendorf", no. 34o1, micro vials: type "Eppendorf", no. 381o, Netheler und Hinz, D-2ooo Hamburg 63 spectrofluorometer: model 65o-1oS, Perkin Elmer GmbH, D-777o Überlingen rotary mixer: Cenco Instrumenten, Breda, The Netherlands pH-meter: PW 94o9, glass electrode: CA/14o2 Philips GmbH, D-35oo Kassel
Chemicals and reagents:	4-methylumbelliferyl-phosphate, no. 4o5663, standard: acid phosphatase (E.C. 3.1.3.2) from potatoes, specific activity: 2 U/mg, no. 1o8219, Boehringer Mannheim GmbH, D-68oo Mannheim 31

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(a) macrophages and granulocytes

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sodium acetate, no. 6267,
E. Merck,
D-61oo Darmstadt 1

glycine, no. 67126,
Sigma Chemie GmbH,
D-8o21 Taufkirchen

chinine sulfate, (standard solution: 1.1 ug/ml o.o5 mol/l sulfuric acid), no. 2-8838,
Carl Roth GmbH,
D-75oo Karlsruhe 21

Procedure:

according to Robinson, D. and Willcox, P., Biochim. Biophys. Acta, 191: 183-186 (1969)

final concentrations of components in assay mixture:

4-methylumbelliferyl phosphate	5o nmol/l
sodium acetate, pH 5.o	25 mmol/l

incubation time: 4o min

fluorometric determination:

excitation wavelength: 365 nm
emission wavelength: 448 nm
slit of excitation and emission monochromators: 2 nm

Calculation:

conversion of fluorescence (Frel) into concentration of reaction product 4-methylumbelliferon by means of pocket calculator program (TI 59 program date: 28.Sep.81, see TABLE ..)

standard curve: see FIGURE ...

Scientific version: 6.Mar.81
Text version: 6.Okt.81

2501091194

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Determination of alanine aminotransferase (glutamate pyruvate transaminase, GPT, EC 2.6.1.2) activity

Principle:	photometric determination of NADH oxidation during the formation of lactate (lactate dehydrogenase, EC 1.1.1.27) from pyruvate
Time	
Sampling:	-
Determination:	-
Sample material and quantity:	serum, 1o ul
Results expressed in:	U/l
Equipment:	ABA-1oo Bichromatic Analyzer, Abbott Laboratories, Diagnostics Division, So. Pasadena, California 91o3o, USA
Chemicals and reagents:	test set GPT, optimized method, no. 161o71, Boehringer Mannheim GmbH, D-68oo Mannheim 31
Procedure:	according to Bergmeyer, H.U. and Bernt, E. in: Bergmeyer, H.U. (ed.): Methoden der enzymatischen Analyse, 3rd edition, Vol. 1, Verlag Chemie Weinheim, 1974, p. 785 photometric determination: wavelenght 1: 34o nm wavelength 2: 38o nm
Scientific version:	1.Sep.78
Text version:	23.Sep.81

2501091195

Source: https://www.industrydocuments.ucsf.edu/docs/lghl0000

Analysis of specific antibody production

Principle:	testing of sera from immunized animals and of culture-conditioned medium from hybridoma cells for specific antibody production against nicotine or cotinine
Time	
Sampling:	-
Determination:	-
Sample material and quantity:	2 ml of rabbit or mouse serum from immunized and non-immunized control animals o.5 ml of hybridoma culture conditioned medium
Results expressed in:	lowest concentration giving a full response noted from serial serum or culture-conditioned medium
Equipment:	PVC microtiter plate, immulon, M 124 A, Cooke Engineering Company, Alexandria, Va. , USA, via Dynatech, D-731o Plochingen liquid scintillation counter or gamma-counter
Chemicals and reagents:	nicotine, no. 77635, Serva Feinbiochemica GmbH und Co., D-69oo Heidelberg cotinine, Labor Dr. R. Hallermayer, D-89oo Augsburg human serum albumin, no. A 8763, Sigma Chemie GmbH, D-8o21 Taufkirchen goat anti-rabbit IgG, no. 65oo, goat anti-mouse IgG, no. 655o, both conjugated with alkaline phosphatase, Medac GmbH, D-2ooo Hamburg 36

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PBS,
Flow Laboratories GmbH,
D-53o9 Meckenheim

fetal calf serum, no. 629,
Difco Laboratories,
Detroit, Michigan, USA

or
goat anti-rabbit IgG, no. 412o and
goat anti-mouse IgG, no. 415o,
radioactively labeled with 125I,
either by radioiodine labeling service,
New England Nuclear, Radioiodination Lab.,
D-6o72 Dreieich

or
by radioiodine labeling using Bolton and Hunter reagent IM. 861,
Amersham Buchler,
D-33oo Braunschweig

p-nitrophenylphosphate, no. 1o4-o,
diethanolamine buffer, no. D 8885,
Tween 2o (polyoxyethylenesorbitan monolanrate), no. P 1379,
Sigma GmbH,
D-8o21 Taufkirchen

biotin-conjugated goat anti-mouse IgG, no. BA-oo6,
biotin-conjugated goat anti-rabbit IgG, no. BA-oo7,
avidin alkaline phosphatase, no. BA-1o5,
Medac GmbH,
D-2ooo Hamburg 36

Procedure:

HSA-conjugated hapten dissolved at 2 ug/ml in PBS, pH 7.2 and addition of 4o ul aliquots into microtiter plate wells. Plates incubated overnight at 4 degrees centigrade

the soluble material collected and stored, the plates washed once with PBS and then incubated with a 1o o/o fetal calf serum/ PBS solution for 3o to 6o min

plates washed 4 to 6 times with PBS

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culture conditioned medium or animal serum supplemented with 1 o/o HSA (in order to bind anti-HSA antibodies) and added at various dilutions (in PBS) to the plates at 5o ul/well

controls received immunesera or culture conditioned media of known specific antibody titer if available (= "high control"), excess amounts of uncoupled unlabeled hapten (nicotine, cotinine) together with test sera (= "low control") sera of non immunized animals (= "low control")

plates incubated for 2 to 24 h

plates washed 4 to 6 times with PBS

for detection of specific antibodies bound to the immunogen possible by radioimmunassay, enzyme-linked immunoassay, biotin/avidin-linked enzyme immunoassay

Radioimmunoassay:

125 I-goat anti-mouse IgG, or 125 I-goat anti-rabbit IgG, added, respectively, at a concentration corresponding to 1oooo to 2oooo cpm/(6o ul x well), together with o.1 o/o Tween 2o

incubation for 2 to 6 h at RT

plates washed 4 times in PBS plus o.1 o/o Tween 2o, single wells cut out with scissors and radioactivity determined with gamma-counter

Enzyme-linked immunoassay:

alkaline phosphatase-linked goat anti-mouse IgG or goat anti-rabbit IgG, respectively, added in 5 serial steps of 1 : 5 dilutions, beginning at 1 : 25, incubated for 2 to 18 h at RT, plates washed 4 times in PBS plus o.1 o/o Tween 2o

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addition of 1oo ul of p-nitrophenylphosphate in 1 mg/ml of 1 mol/l diethanolamine buffer, pH 9.8, incubation for 15 min at RT

reaction stopped by addition of 25 ul of 2 mol/l NaOH

yellow precipitate detected or measured photometrically at 4o5 nm

Biotin/avidin-linked immunoassay:

addition of biotin-conjugated goat anti-mouse or goat anti-rabbit IgG, respectively, incubation overnight, 4 to 6 washes with PBS

addition of alkaline phosphatase-conjugated avidin, incubation for 1 h at RT, 4 to 6 washes with PBS

proceeding with alkaline phosphatase reaction : see enzyme-linked immunoassay

Scientific version: 15.Dec.8o
Text version: 12.Oct.81

2501091199

Source: https://www.industrydocuments.ucsf.edu/docs/lghl0000

Determination of "arterial" pressure

Principle:	determination of displacement of sensing diaphragm by transducing element
Time:	continuously during perfusion
Sample material:	"arterial" perfusate, site of determination: see FIGURE .
Results expressed in:	kPa
Equipment:	Statham pressure registering dome P 23 Db, pressure coupler type 551 A, Watanabe recorder (3 channels) WTR 281, Hugo Sachs Elektronik KG, D-78o1 March-Hugstetten/Freiburg
Chemicals and reagents:	9 g/l sodium chloride, no. 64o4, E. Merck, D-61oo Darmstadt 1
Scientific version: Text version:	17.Mar.8o 21.Sep.81

2501091200

Source: https://www.industrydocuments.ucsf.edu/docs/lghl0000

Determination of aryl hydrocarbon monooxygenase (EC 1.14.14.2) activity

Principle:	fluorometric determination of 3- and 9-OH-B(a)P formed during the incubation of B(a)P with tissue extracts and separated after incubation by HPLC
Time	
Sampling:	on day ...
Determination:	within ... .. after preparation of microsomes or S9 fraction
Sample material and quantity:	epidermis: 4o to 14o ug microsomal protein kidneys : o.1 - o.3 mg microsomal protein, linear up to o.3 mg protein liver : S9 fraction, approx. 1o ug of protein lung : o.15 - o.3 mg microsomal protein, linear up to o.4 mg protein tracheal mucosa : ....
Results expressed in:	U/mg protein (1 U = 1 nmol 3- and 9-OH-B(a)P/h)
Equipment:	rotary mixer: Cenco Instrumenten, Breda, The Netherlands high pressure liquid chromatograph: model 1o84 B, Hewlett Packard, D-75oo Karlsruhe column: RP-18, 25o mm x 4.6 mm, Knauer, D-637o Oberursel incubation vessels: no. 611-52, Sovirel, Le Vallois Perret, France

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injection vial: no. 8o184, 11.5 mm x 325 mm,
Müller und Müller Glaswarenfabrik,
D-345o Holzminden

spectrofluorometer: model 65o-1oS,
Perkin Elmer GmbH,
D-777o Überlingen

shaking water bath: no. 3o47,
Köttermann,
D-3165 Hänigsen

centrifuge: RC-5B, rotor: SS-34,
Du Pont Instruments,
D-635o Bad Nauheim

Chemicals and reagents:

magnesium chloride, no. 5833,
acetonitrile, no. 3o,
monosodium phosphate buffer, pH 4.8, no. 6346,
acetone, no. 14,
E. Merck,
D-61oo Darmstadt 1

glucose-6-phosphate dehydrogenase (G6PDH, EC 1.1.1.49), no. 197726,
glucose-6-phosphate (G6P), no. 127o27,
NADP, no. 128o31,
NAD, no. 127981,
Boehringer Mannheim GmbH,
D-68oo Mannheim 31

benzo(a)pyrene (B(a)P), no. 3176,
chinine sulfate (standard solution: 1.1 ug/ml o.o5 mol/l sulfuric acid), no. 2-8838,
Carl Roth GmbH,
D-75oo Karlsruhe 21

bovine serum albumin (BSA), no. A 4378,
tris(hydroxymethyl)aminomethane (Tris), no. T 15o3,
Sigma Chemie GmbH,
D-8o21 Taufkirchen

2501091202

Source: https://www.industrydocuments.ucsf.edu/docs/lghl0000

Procedure

Enzyme incubation: according to Van Cantfort, J., De Graeve, J. and Gielen, J.E., Biochem. Biophys. Res. Commun. 79: 5o5-512 (1977)

incubation time:

epidermis	3o min
kidneys	15 min
liver	1o min
lung	25 min
tracheal mucosa	.. min

final concentrations of components in assay mixture:

microsomal protein	... g/l
B(a)P	o.o8 mmol/l
NAD	o.43 mmol/l
NADP	o.37 mmol/l
G6P	2.5o mmol/l
BSA	o.8o g/l
Tris-HCl, pH 7.6	5o.oo mmol/l
magnesium chloride	5.oo mmol/l
G6PDH	1 U/ml

triplicate determination per sample

HPLC separation

Injection volume: 2o ul

Column: RP-18, particle diameter: 1o um, column length: 25o mm, inner diameter: 4.6 mm

Oven and solvent temperature: 4o degrees centigrade

Solvent A: 5oo ml/l acetonitrile
5oo ml/l 1o mmol/l sodium dihydrogen phosphate buffer, pH 4.8

Solvent B: 1ooo ml/l acetonitrile

Gradient: linear increase from 8oo ml/l A and 2oo ml/l B to 1oo ml/l A and 9oo ml/l B within 2o min

resulting gradient:
6oo to 95o ml/l acetonitrile

Flow rate: o.8 ml/min

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Column pressure:	7o to 1oo bar
Detection:	fluorometric, excitation wavelength: 3o5 nm, emission wavelength: 43o nm, slit of excitation and emission monochromators: 15 nm
Calculations:	standard curve: see FIGURE ...

Scientific version: 4.Feb.81
Text version: 2o.Aug.81

2501091204

Source: https://www.industrydocuments.ucsf.edu/docs/lghl0000

Determination of aspartate aminotransferase (glutamate oxalo-acetate transaminase, GOT, EC 2.6.1.1) activity

Principle:	photometric determination of the NADH oxidation during the formation of malate (malate dehydrogenase, EC 1.1.1.37) from oxaloacetate
Time	
Sampling:	-
Determination:	-
Sample material and quantity:	serum, 1o ul
Results expressed in:	U/l
Equipment:	ABA-1oo Bichromatic Analyzer, Abbott Laboratories, Diagnostics Division, So. Pasadena, California 91o3o, USA
Chemicals and reagents:	test set GOT, optimized method, no. 158178, Boehringer Mannheim GmbH, D-68oo Mannheim 31
Procedure:	according to Bergmeyer, H.U. and Bernt, E. in: Bergmeyer, H.U. (Ed.): Methoden der enzymatischen Analyse 3rd ed., Vol. 1, Verlag Chemie Weinheim, 1974, p. 769 photometric determination: wavelength 1: 34o nm wavelength 2: 38o nm
Scientific version:	1.Sep.78
Text version:	23.Sep.81

2501091205

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Determination of bilirubin concentration

Principle:	photometric determination of azo-bilirubin formed by the reaction of bilirubin with the diazonium salt of 2.4-dichloroaniline in the presence of sulfanilic acid and 5o o/o methanol
Time	
Sampling:	-
Determination:	-
Sample material and quantity:	serum, 1o ul
Results expressed in:	umol/l
Equipment:	ABA-1oo Bichromatic Analzyer, Abbott Laboratories, Diagnostics Division, So. Pasadena, California 91o3o, USA
Chemicals and reagents:	test set Bilirubin A-Gent, no. 6o42, Abbott Laboratories, Diagnostics Division, So. Pasadena, California 91o3o, USA
Procedure:	according to Malloy, H.T., Evelyn, K.A., J. Biological Chemistry 119: 481 (1937) with modifications by Rand, R.V., di Pasqua, A., Clinical Chemistry 8: 57o (1962) and Abbott Diagn. photometric determination: wavelength 1: 55o nm wavelength 2: 65o nm
Scientific version:	1.Sep.78
Text version:	21.Sep.81

2501091206

Source: https://www.industrydocuments.ucsf.edu/docs/lghl0000

Blood gas analysis

Principle:	(1) polarographic determination of oxygen partial pressure of venous blood (2) electrochemical determination of hydrogen ion concentration of venous blood (3) electrochemical determination of hydrogen ion concentration of 2 venous blood samples, equilibrated with defined carbon dioxide partial pressure and graphical determination of the sample carbon dioxide partial pressure, standard bicarbonate, base excess and buffer base using a Siggaard-Andersen nomogram.
Time	
Sampling:	-
Determination:	-
Sample material and quantity:	venous blood, heparinized, 4oo ul
Results expressed in:	(1) oxygen partial pressure: mm Hg (2) hydrogen ion concentration: pH-units (3) carbon dioxide partial pressure: mm Hg standard bicarbonate: mmol/l base excess: mmol/l buffer base: mmol/l
Equipment:	Astrup Micro Equipment, model AME 1, Radiometer A/S, DK-24oo Copenhagen, Denmark heparinized (o.55 mg/ml) capillary tube, no. D 551-1o-8o, Radiometer A/S, DK-24oo Copenhagen, Denmark

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	sealing wax, D558, Radiometer A/S, DK-2400 Copenhagen, Denmark
Chemicals and reagents:	oxygen-zero solution, no. S4150, calibration buffer: pH 6.841, no. S1500, calibration buffer: pH 7.383, no. S1510, Radiometer A/S, Dk-2400 Copenhagen, Denmark
Procedure:	acc. to Clark, L.C., Tr. Am. Soc. for Art. Int. Organs 2: 41 (1956), Siggaard-Andersen, O. and Engel, K., Scand. J. Clin. Lab. Invest. 12: 177 (1960), Siggaard-Andersen, O., Scand. J. Clin. Lab. Invest. 14: 598 (1962), Pfefferkorn, B., Z. Versuchstierk. 12: 363 (1970) and manufacturers manual
Scientific version:	8.Oct.80
Text version:	21.Sep.81

2501091208

Source: https://www.industrydocuments.ucsf.edu/docs/lghl0000

Blood sampling

Principle:	puncture of retroorbital vein plexus during anesthesia
Time	
Sampling:	-
Results expressed in:	-
Sample material and quantity:	blood, approx. 1 ml
Equipment:	glass capillary: length: approx. 5 cm outer diameter: 1.4 mm Drummond Microcaps via Faust GmbH, D-5ooo Köln 9o micro vials: type "Eppendorf", polypropylene, no. 381o, Netheler und Hinz, D-2ooo Hamburg 63
Chemicals and reagents:	diethyl ether
Procedure:	insertion of capilary into retro-orbital vein plexus usually at right eye, left plexus used, when lesions observed at right eye collection of blood in micro vials storage at RT
Scientific version: Text version:	22.Apr.8o 21.Sep.81

2501091209

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Blood sampling and serum preparation

Principle:	(1) rabbits: incomplete bleeding by ear vein fission. Collection of serum after glass bead defibrination (2) mice: complete bleeding from decapitation wound
Time	
Sampling:	-
Determination:	-
Sample material and quantity:	(1) immunized rabbits, (2) non-immunized control rabbits, (3) immunized BALB/c mice, (4) non-immunized BALB/c mice,
Results expressed in:	-
Equipment:	syringe for blood collection: Sicherheits-Monovette, 5 ml, no. o5.o71, filter assembly for serum separation: Ventilfilter, no. 53.43o, Sarstedt, D-5223 Nümbrecht razor-blade
Chemicals and reagents:	ethanol, 7oo ml/l, no. 972, E. Merck, D-61oo Darmstadt 1
Procedure	
Rabbits:	inner side of the ear rinsed with ethanol, major ear vein cross-sectioned with razor-blade, blood drawn into syringe. Syringe shaken with glass beads for 15 min to precipitate fibrin at the beads. Defibrinated blood transferred into

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	the filter assembly, centrifuged at 3.9x1oE4 m/s2 (= 4ooo x g) for 1o min and serum collected by depression of filter assembly according to the manufacturer's description
	serum stored at minus 2o degrees centigrade
Mice:	decapitation, collection of blood from the opened blood vessels, further processing of blood as described above

Scientific version: Oct.8o
Text version: 8.Oct.81

2501091211

Source: https://www.industrydocuments.ucsf.edu/docs/lghl0000

Determination of body weight

Principle:	gravimetric determination
Time:	immediately prior to narcotization
Sample material and quantity:	rats, individually
Results expressed in:	g
Equipment:	digital animal scales: no. 3716, Sartorius GmbH, D-34oo Göttingen
Chemicals and reagents:	-
Procedure:	gravimetric determination of individual body weight
Scientific version:	3o.Jan.8o
Text version:	24.Sep.81

2501091212

Source: https://www.industrydocuments.ucsf.edu/docs/lghl0000

Determination of body weight

Principle:	gravimetric determination
Time:	immediately before killing
Sample material and quantity:	rats, 1 to 2o
Results expressed in:	g
Equipment:	animal scales: no. 12717, Toledo Werk GmbH, D-5ooo Köln 41
Chemicals and reagents:	-
Procedure:	weighing of rats in inhalation tube boards calculation of mean body weight: BW = (weight of board with N rats minus weight of board without rats)/N
Scientific version:	16.Sep.8o
Text version:	24.Sep.81

2501091213

Source: https://www.industrydocuments.ucsf.edu/docs/lghl0000

Determination of body weight

Principle:	gravimetric determination
Time:	immediately before each administration of immunogen, all rabbits or mice individually
Sample material and quantity:	rabbits or mice
Results expressed in:	g
Equipment:	digital animal scales: no. 3716, Sartorius GmbH, D-34oo Göttingen
Chemicals and reagents:	-
Procedure:	gravimetric determination of individual body weight with time integration to correct for movement of rabbits or mice
Scientific version:	Oct.8o
Text version:	24.Sep.81

2501091214

Source: https://www.industrydocuments.ucsf.edu/docs/lghl0000

Cell counting (hemocytometer-method)

Principle:	microscopic determination of stained cells in defined volume of hemocytometer
Time	
Sampling:	on day ...
Determination:	immediately after harvest of cells
Sample material and quantity:	free lung cells suspension, approx. 5 ul
Results expressed in:	(1) 1oE5 macrophages/.. (2) 1oE5 granulocytes/..
Equipment:	Bürker hemocytometer chamber (height of counting chamber: o.1 mm), no. 9161o89, Hirtz und Co., D-5ooo Köln 51 microscope: 4oo-fold magnification, standard 14, objective: PL 4o/o.65, Carl Zeiss, D-7o82 Oberkochen micro vials: type "Eppendorf", polypropylene, no. 381o, Netheler und Hinz, D-2ooo Hamburg 63 Coulter mixer C5, Coulter Electronics Limited, D-415o Krefeld
Chemicals and reagents:	Türk's staining reagent: o.2 g/l gentiana violet, 64 ml/l acetic acid, E. Merck, D-61oo Darmstadt 1
Procedure:	5o ul of suspended cells mixed in micro vial with 5o ul of Türk's staining reagent. After 1 min staining at RT sample introduced into the hemocytometer chamber.

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Source: https://www.industrydocuments.ucsf.edu/docs/lghl0000

3 to 9 square-fields counted under a microscope. Calculation of the number of macrophages and granulocytes.

Scientific version: 7.Feb.79
Text version: 28.Sep.81

2501091216

Source: https://www.industrydocuments.ucsf.edu/docs/lghl0000

Cell culture

Principle:	growth of mouse cells under sterile conditions: suspension culture of primary lymphocytes and permanent myeloma cell line
Time:	-
Sample material and quantity:	(1) primary cultures of spleen lymphocytes (2) permanent cultures of myeloma cells for fusion (3) permanent hybridoma cell lines as feeder cells
Equipment:	petri dishes, bacterial, diameter: 9.4 cm, no. 6331o2, Greiner GmbH, D-744o Nürtingen incubator: B 5o/6o, W.C. Heraeus GmbH, D-645o Hanau 1
Chemicals and reagents:	growth medium R1o: RPMI 164o medium, Seromed GmbH, D-8ooo München 71 supplemented with 1o o/o inactivated fetal calf serum, no. 29-1o1-54, Penicillin (1oo ug/ml) plus Streptomycin (1oo ug/ml), no. 16-7oo-49, Flow Laboratories GmbH, D-53o9 Meckenheim 2-mercaptoethanol 5x1oE-5 mol/l, no. 8o574o, E. Merck, D-61oo Darmstadt 1

Procedure

Primary lymphocyte cultures:	kept in culture at approx. 5x1oE5 to 1x1oE6 cells/ml

2501091217

Source: https://www.industrydocuments.ucsf.edu/docs/lghl0000

Permanent cell line:	P3/X63-Ag8.653 (short name: Ag8) mouse myeloma from strain BALB/c is a non-secreting derivative of the P3/X63-Ag8 line (a), originating from the laboratory of Dr. C. Milstein, Cambridge, the variant being selected by Drs. J.F. Kearney, A. Radbruch and B. Liesegang, Institute for Genetics, University of Cologne (b).
	permanent cells grown in R1o medium in bacterial petri dishes, inoculated at an initial cell density of approx. 1x1oE4 cells/ml and grown until confluency. Cells subcultured by dilution with R1o medium. Stocks of these cells kept in liquid nitrogen
	both cell types grown in a humidified incubator with 5 o/o CO2 atmosphere at 37 degrees centigrade
Scientific version:	8.Jan.81
Text version:	28.Sep.81

2501091218

(a) This cell line is genetically deficient for the enzyme hypoxanthine-guanosine-phosphoribosyl transferase (HGPRT) (EC 2.4.2.8), which serves as a negative selection marker.
(b) Kind donation we herewith acknowledge.

Source: https://www.industrydocuments.ucsf.edu/docs/lghl0000

Cell fusion and selection

Principle:	fusion of different cell types by aid of polyethylene glycol to form new hybrid cells with combined phenotypes chemical selection to kill the non-fused myeloma cells and to propagate hybrid cells (Littlefield, 1964)
Time:	-
Sample material and quantity:	lymphocytes and myeloma cells
Equipment:	prefilter, no. 63-735-o4, Flow Laboratories GmbH, D-53o9 Meckenheim 5o ml polypropylene test tube, no. 2o7o, Falcon, Oxnard, USA Costar Cluster 96 plates, no. 3596, Costar via Tecnomara AG, D-6331 Hüttenberg/Giessen
Chemicals and reagents:	phosphate buffered saline (PBS), Flow Laboratories GmbH, D-53o9 Meckenheim polyethylene glycol, Mr 4ooo, no. 2-o156, Carl Roth GmbH, D-75oo Karlsruhe 21 dimethyl sulfoxide (DMSO), no. 9678, E. Merck, D-61oo Darmstadt 1 polyethylene glycol preparation: 2o g PEG dissolved in 28 ml sterile PBS, containing 15 o/o DMSO and mixture heated up to 6o degrees centigrade. Sterilisation by membrane filtration

2501091219

Source: https://www.industrydocuments.ucsf.edu/docs/lghl0000

"HAT"-medium: R 1o medium supplemented with hypoxanthine (final concentration 13.61 mg/l), aminopterin (191.o ug/l), thymidine (382.5 ug/l), made up from a 5ox concentrated stock solution, HAT 5ox, no. 73-151-49, Flow Laboratories GmbH, D-53o9 Meckenheim

Procedure

Fusion:

(1) lymphocytes and myeloma cells pelleted by centrifugation (9.81x1oE3 m/s2 (= 1ooo x g) for 1o min) and washed 1 time in PBS, counted and mixed at a ratio of 1o : 1 up to 1o : 5, repeated centrifugation, thereafter complete removal of supernatant, tube flicked with the finger in order to loosen the pellet

(2) addition of 1.5 ml prewarmed (37 degrees centigrade) PEG solution, cells resuspended by gentle shaking and kept for 1 min at 37 degrees centigrade in waterbath

(3) cell solution diluted by dropwise addition of 5 ml PBS (RT) under gentle shaking, addition of another 5 ml PBS in one step, centrifugation (9.81x1oE3 m/s2 (= 1ooo x g) for 1o min), and removal of supernatant

Selection:

(1) cell pellet resuspended in 4.5 ml HAT medium per 1oE8 cells by careful addition of medium with pipette in order to prevent breaking of all clumps, 1 drop of cell suspension added with 1 ml pipette to each well of a 24 well tissue culture plate

containing 2.5 ml of HAT medium (prewarmed in a CO2 incubator). 1oE8 fused cells resuspended in 4.5 ml HAT medium and distributed into the wells of 4 tissue culture plates, 1oE6 cells/well

Source: https://www.industrydocuments.ucsf.edu/docs/lghl0000

(2) addition of feeder cells: either 3x1oE6 thymocytes (prepared as spleen cells) or 3x1oE6 spleen cells per well kept from 1/1o of the spleen cells before fusion added to fused cells to improve the hybridoma yield

feeding of cells 1 time per week by removal of 1 ml of medium and addition of 1 ml fresh HAT medium

after 2 to 4 weeks growth of cells detectable with the bare eye, cultures fed approx. 2 times with HAT medium before adjustment to normal growth medium (R1o).

after repeated fusion initial cultivation done at a cell density of growth in .LT. 5o o/o of all wells (often o.5x1oE6 cells/well or less sufficient)

Scientific version: 15.Dec.8o
Text version: 28.Sep.81

2501091221

Source: https://www.industrydocuments.ucsf.edu/docs/lghl0000

Determination of cell viability (Trypanblue-method)

Principle:	viable cells exclude ionic dyes such as Trypanblue during the first five minutes of incubation from the cytoplasm
Time	
Sampling:	on day ..
Determination:	within .. hours after start of harvest of cells
Sample material and quantity:	free lung cells, 5 ul
Results expressed in:	percent viable (= not immediately diffusely stained) cells
Equipment:	Bürker hemocytometer chamber (height of counting chamber: o.1 mm), no. 9161o89, Hirtz und Co., D-5ooo Köln 51 microscope: 4oo-fold magnification, standard 14, objective: PL 4o/o.65, Carl Zeiss, D-7o82 Oberkochen micro vials: type "Eppendorf", polypropylene, no. 381o, Netheler und Hinz, D-2ooo Hamburg 63 Coulter mixer C5, Coulter Electronics Limited, Hertfordshire, United Kingdom
Chemicals and reagents:	dye solution: 5 g/l Trypanblue in cell culture medium, no. 1B187, Chroma Gesellschaft Schmidt und Co., D-7ooo Stuttgart-Untertürkheim cell culture medium: Eagle's Essential basic medium, 1o-fold concentrate, no. 14-33o-54, Flow Laboratories GmbH, D-53o9 Meckenheim,

2501091222

Source: https://www.industrydocuments.ucsf.edu/docs/lghl0000

with sodium bicarbonate, Hepes, glutamin and Penicillin/Streptomycin final pH 7.4, 3o4 mmol/kg

Procedure:

cell suspension mixed with equal volume of dye solution, cells counted after 5 minutes of incubation at o degrees centigrade

number of counted cells: approx. 1oo

Scientific version: 1.Jul.8o
Text version: 12.Oct.81

2501091223

Source: https://www.industrydocuments.ucsf.edu/docs/lghl0000

Determination of cholesterol concentration

Principle:	photometric determination of the red product formed between 4-aminophenazone, phenol and hydrogen peroxide which is produced by the cholesterol-oxidase (EC 1.1.3.6) reaction from cholesterol after hydrolysis (cholesterolesterase, EC 3.1.1.13) of serum cholesterol esters
Time	
Sampling:	-
Determination:	-
Sample material and quantity:	serum, 5 ul
Results expressed in:	mmol/l
Equipment:	ABA-1oo Bichromatic Analyzer, Abbott Laboratories, Diagnostics Division, So. Pasadena, California 91o3o, USA
Chemicals and reagents:	test set Cholesterol CHOD-PAP, no. 172 626, Boehringer Mannheim GmbH, D-68oo Mannheim 31
Procedure:	according to Schettler, G, Nüssel, E., Arbeitsmed., Sozialmed., Präventivmed. 1o: 25 (1975) photometric determination: wavelength 1: 5oo nm wavelength 2: 6oo nm
Scientific version:	1.Sep.78
Text version:	21.Sep.81

2501091224

Source: https://www.industrydocuments.ucsf.edu/docs/lghl0000

Determination of citrate concentration

Principle:	fluorometric determination of NADH oxidized during the formation of malate (malate dehydrogenase, EC 1.1.1.37) from oxaloacetate which is obtained from citrate in a reaction catalyzed by citrate lyase (EC 4.1.3.6). Pyruvate, which is formed by spontaneous decarboxylation of oxaloacetate, is also oxidized in the presence of lactate dehydrogenase (EC 1.1.1.27).
Time	
Sampling:	-
Determination:	within ... .. after sampling
Sample material and quantity:	muscle tissue extract, deproteinized
Results expressed in:	nmol/g WW
Equipment:	spectrofluorometer: model 65o-1oS, Perkin Elmer GmbH, D-777o Überlingen
Chemicals and reagents:	Tris (hydroxymethylamino) methane, no. 8382, magnesium chloride, no. 5886, ethylenediaminetetraacetic acid, no. 8418, E. Merck, D-61oo Darmstadt 1 NADH, disodium salt, no. 1o7727, malate dehydrogenase (EC 1.1.1.37), no. 127256, lactate dehydrogenase (EC 1.1.1.27), no. 1o68984, citrate lyase (EC 4.1.3.6), no. 1o3365, Boehringer Mannheim GmbH, D-68oo Mannheim 31

2501091225

Source: https://www.industrydocuments.ucsf.edu/docs/lghl0000

Procedure:	according to Passonneau, J.V., Brown, J.G., in: Bergmeyer, H.U. (ed.): Methoden der enzymatischen Analyse, Vol. 2, Verlag Chemie Weinheim, 3rd edition, 1974, pp. 1611-1615
	fluorometric determination:
	wavelength of excitation: 36o nm wavelength of emission: 46o nm
	storage until determination: minus 2o degrees centigrade
Scientific version:	16.Apr.8o
Text version:	25.Jun.81

2501091226

Source: https://www.industrydocuments.ucsf.edu/docs/lghl0000

Cloning

Principle:	screening for enrichment and isolation of antigen-specific antibody secreting cells, growth and selection of hybridoma clones out of single cells, supported by addition of feeder cells
Sample material and quantity:	cell inoculum per large well (see below): approx. 1x1oE4 cells, in 2 ml growth medium R1o as feeder cells either: (1) freshly prepared BALB/c spleen cells (1x1oE6 untreated cells/well) (see .....), or (2) peritoneal BALB/c mouse macrophages, washed out from the peritoneal cavity, (3x1oE3 macrophages/well), or (3) hybridoma cells (22.5.8o-14 AF), produced by J.H. Peters, from stocks kept in liquid nitrogen. before inoculation cells treated with 3o ug/ml Mitomycin C for 2o min
Results expressde in:	-
Equipment:	tissue culture plates 384 (every tissue culture plate divided into 24 large wells with 16 small wells inside each), no. 7o416o, Greiner GmbH, D-744o Nürtingen Costar Cluster 96 plates, no. 3596, Costar via Tecnomara AG, D-6331 Hüttenberg/Giessen
Chemicals and reagents:	Agar Noble, no. o142-o2-7, Difco Laboratories, Detroit, Michigan, USA, via Nordwald KG, D-2ooo Hamburg 5o Mitomycin C, no. 298o5, Serva Feinbiochemica GmbH, D-69oo Heidelberg 1

2501091227

Source: https://www.industrydocuments.ucsf.edu/docs/lghl0000

growth medium R1o:
RMPI 164o medium:
Seromed,
D-8ooo München 71

supplemented with 1o o/o inactivated fetal calf serum, no. 29-1o1-54, Penicillin (1oo ug/ml) plus Streptomycin (1oo ug/ml), no. 16-7oo-49, Flow Laboratories GmbH, D-53o9 Meckenheim

and
2-mercaptoethanol, 5x1oE-5 mol/l, no. 8o574o, E. Merck, D-61oo Darmstadt 1

Procedure:

hybridoma cells growing after HAT selection transferred into tissue culture plates, consisting of large wells, each of them subdivided into small wells

after 2 to 4 days of growth 1oo ul of conditioned growth medium collected from each well and tested for specific antibodies (see .....). In positive wells the medium level lowered so that the smaller septa form 16 individual small wells. After 1 day of culture, conditioned growth media from individual small wells tested again for antibody secretion, positive cultures processed for cloning. Conditioned medium from productive and non-productive growing cultures collected and stored at plus 4 degrees centigrade until used for cloning

for cloning positive cultures diluted to a cell suspension containing 5 cells/ml, R1o medium supplemented with 5o percent conditioned medium prepared from growing cultures. The cells seeded into cluster 96 plates at o.2 ml/well (avarage 1 cell/well) together with feeder cells

Scientific version: 26.Dec.8o 2501091228
Text version: 23.Sep.81

Source: https://www.industrydocuments.ucsf.edu/docs/lghl0000

Collection of free lung cells

Principle:	centrifugation
Time	
Sampling:	on day ...
Determination:	-
Sample material and quantity:	total lavage fluid recovered from lung, (1) individual rats (2) .. rats pooled
Results expressed in:	-
Equipment:	centrifuge: model J6, rotor: JS-4.2, Beckmann Instruments GmbH, D-8ooo München 4o pasteur pipettes: silanized, no. H 55698, Harshaw, De Meern, The Netherlands test tubes: no. 2o7o, conical graduated, polypropylene, diameter: 3o mm, length: 115 mm, Falcon, Div. Becton and Dickinson GmbH, D-69oo Heidelberg-Wieblingen
Chemicals and reagents:	cell suspension medium: medium 199 (modified), with Earle's salts, without sodium bicarbonate, without glutamine, with 2o mmol/l Hepes buffer and addition of Penicillin and Streptomycin, no. 12-2o4-49, Eagle's essential medium, according to Eagle, H., Science 13o: 342 (1959), 1o-fold concentrate, no. 14-33o-54, Flow Laboratories GmbH, D-53o9 Meckenheim

2501091229

Source: https://www.industrydocuments.ucsf.edu/docs/lghl0000

Procedure

Storage:	until centrifugation at o degrees centigrade
Centrifugation:	4.9x1oE3 m/s2 (= 5oo x g), for 1o min, o to 5 degrees centigrade
Removal of supernatant:	from cell pellet with suction pump and pasteur pipettes
Resuspension of cells:	for determination of phagocytic index with medium 199 and for determination of phagoluminescence with Eagle's essential medium by trituration with silanized pasteur pipettes

Scientific version: 16.Sep.8o
Text version: 5.Oct.81

2501091230

Source: https://www.industrydocuments.ucsf.edu/docs/lghl0000

Conjugation of haptens to carrier protein (preparation of immunogen)

Principle:	peptide bond formation between carboxyl group of haptens and epsilon-aminogroups of human serum albumin lysine residues in the presence of a carbodiimide. Reaction may be represented as a dehydration, but the mechanism is not fully understood
Time:	-
Sample material and quantity:	3'-SMN, 1o mg 4'-COC, 1o mg human serum albumin, 1o mg
Results expressed in:	-
Equipment:	chromatography column diameter: 1.5 cm height of gel: approx. 35 cm fraction collector: Ultro Rac 2 model 2o7o, UV monitor: Uvicord 3 model 2o89, recorder: potentiometric 1-channel-recorder model 221o, LKB Instrument GmbH, D-8o32 Gräfelfing
Chemicals and reagents:	1-ethyl-3-(dimethylaminopropyl)carbodiimide hydrochloride, no. E 775o, Sigma Chemie GmbH, D-8o21 Taufkirchen sodium chloride, no. 64o4, sodium di-hydrogen phosphate, no. 6346, di-sodium hydrogen phosphate, no. 658o, E. Merck, D-61oo Darmstadt 1

2501091231

Source: https://www.industrydocuments.ucsf.edu/docs/lghl0000

Sephadex G-5o, coarse,
Pharmacia Fine Chemicals,
D-78oo Freiburg 1

human serum albumin, essentially globulin free, no. A 8763,
Sigma Chemie GmbH,
D-8o21 Taufkirchen

Procedure

Conjugation:	o.5 ml hapten solution (2o mg/ml water) plus o.5 ml albumin solution (2o mg/ml water), adjustment of pH with o.1 mol/l phosphate buffer (pH 7.o) to approx. pH 7.o, addition of 1o mg 1-ethyl-3-(dimethylaminopropyl) carbodiimide hydrochloride, shaking overnight at 25 degrees centigrade
Chromatography of the reaction mixture:	gel: Sephadex G-5o elution medium: 9 g/l sodium chloride in phosphate buffer, pH 7.o fractionation of the column eluate: 1.5 to 2.5 ml per fraction conjugate-peak-detection: 28o nm
Identification of the reaction products:	UV-spectroscopy

Scientific version: 9.Jan.81
Text version: 24.Sep.81

2501091232

Source: https://www.industrydocuments.ucsf.edu/docs/lghl0000

Determination of creatinine concentration

Principle:	photometric determination of orange complex which is formed between creatinine and picrate in alkaline solution
Time	
Sampling:	-
Determination:	-
Sample material and quantity:	(1) perfusate, deproteinized (trichloroacetic acid), 25 ul (2) serum, 25 ul
Results expressed in:	(1) umol/l (2) mmol/l
Equipment:	ABA-1oo Bichromatic Analyser, Abbott Laboratories, Diagnostics Division, So. Pasadena, California 91o3o, USA
Chemicals and reagents:	test set Creatinine, no. 124192, standard: Precinorm, no. 125121, Boehringer Mannheim GmbH, D-68oo Mannheim 31
Procedure:	acc. to Jaffe', Z. Physiol. Chem. 1o: 391 (1886), modified according to Popper, H. et al., Biochem. Z. 291: 354 (1937) photometric determination: wavelength 1: 5oo nm wavelength 2: 6oo nm
Scientific version:	16.Apr.8o
Text version:	24.Sep.81

2501091233

Source: https://www.industrydocuments.ucsf.edu/docs/lghl0000

Determination of creatine kinase (ATP: creatine N-phosphotransferase, EC 2.7.3.2)

Principle:	photometric determination of NADPH formed during the oxidation of glucose-6-phosphate (glucose-6-phosphate-dehydrogenase, EC 1.1.1.49), which is obtained by phosphorylation of glucose with ATP (hexokinase, EC 2.7.1.1). ATP in turn is formed during the creatine kinase catalyzed cleavage of creatine phosphate.
Time	
Sampling:	-
Determination:	-
Sample material and quantity:	(1) perfusate supernatant, 2o ul (2) muscle tissue homogenate, native, 2o ul
Results expressed in:	U/l
Equipment:	ABA-1oo Bichromatic Analyzer, Abbott Laboratories, Diagnostics Division, So. Pasadena, California 91o3o, USA
Chemicals and reagents:	test set CK NAC, activated, no. 181188, standard: Precinorm E, no. 125o91, Boehringer Mannheim GmbH, D-68oo Mannheim 31
Procedure:	acc. to Szasz, G., Gruber, W. and Bernt, E., Clin. Chem. 22: 65o (1976) photometric determination: wavelength 1: 38o nm wavelength 2: 34o nm temperature: 25 ± o.1 degrees centigrade
Scientific version:	5.Feb.8o
Text version:	23.Sep.81

2501091234

Source: https://www.industrydocuments.ucsf.edu/docs/lghl0000

Determination of creatine phosphate, glucose-6-phosphate and ATP concentration

Principle:	photometric determination of NADPH formed during the oxidation of glucose-6-phosphate, which is obtained by phosphorylation of glucose with ATP (hexokinase, EC 2.7.1.1). ATP in turn is formed from creatine phosphate and ADP (creatine kinase, EC 2.7.3.2)
Time	
Sampling:	-
Determination:	within ... .. after sampling
Sample material and quantity:	muscle tissue (a) extract, deproteinized
Results expressed in:	umol/g WW
Equipment:	spectrophotometer: model 555, Perkin Elmer GmbH, D-7770 Überlingen
Chemicals and reagents:	triethanolamine hydrochloride, no. T 1502, Sigma Chemie GmbH, D-8021 Taufkirchen magnesium chloride, no. 5886, glucose, no. 8346, E. Merck, D-6100 Darmstadt 1 NADP, disodium salt, no. 107727, ADP, no. 127507, glucose-6-phosphate dehydrogenase (EC 1.1.49), no. 127043, hexokinase (EC 2.7.1.1), no. 127175, creatine kinase (EC 2.7.3.2), no. 126969, Boehringer Mannheim GmbH, D-6800 Mannheim 31

2501091235

(a) predominantly M. gastrocnemius

Source: https://www.industrydocuments.ucsf.edu/docs/lghl0000

Procedure:	according to Lamprecht, W., Stein, P., Heinz, F., Weisser, H., in: Bergmeyer, H.U. (ed.): Methoden der enzymatischen Analyse, Vol. 2, Verlag Chemie Weinheim, 3rd edition, 1974, pp. 1611-1615 photometric determination: wavelength: 334 nm storage until determination: minus 2o degrees centigrade
Scientific version:	16.Apr.8o
Text version:	7.Okt.81

2501091236

Source: https://www.industrydocuments.ucsf.edu/docs/lghl0000

PART	RESEARCH TEAM	STATE OF ISSUE
1	Analytical Chemistry	-
2	Animal Treatment	-
3	Biological Chemistry	12.Oct.81
4	Cell Biology	-
5	Inhalation, barrier	-
6	Microbiology	-
7	Pharmacology	-
8	Pathology	-

TABLE

METHOD COMPILATIONS

2501091237

Source: https://www.industrydocuments.ucsf.edu/docs/lghl0000

Determination of density

Principle:	determination of refractive index
Time	
Sampling:	on day ...
Determination:	within ... h after start of harvest of free lung cells
Sample material and quantity:	(1) fractionated free lung cells, approx. 5o ul (2) sucrose, approx. 5o ul
Results expressed in:	kg/l
Equipment:	refractometer: Abbé type, model A, Carl Zeiss, D-7o82 Oberkochen thermostat: type RM3, Messgerätewerk Lauda, Dr. R. Wobser KG, D-697o Lauda-Königshofen
Chemicals and reagents:	-
Procedure:	optical determination of refractive index with Abbé type prism refractometer at 2o degrees centigrade calculation of density from standard curve: see TABLE ...
Scientific version:	2.May 79
Text version:	17.Jul.81

2501091238

Source: https://www.industrydocuments.ucsf.edu/docs/lghl0000

Separation of different cell types by density gradient centrifugation

Principle:	separation of cells according to their density during centrifugation in a colloidal silica sol density gradient
Time	
Sampling:	on day ...
Determination:	.. h after harvest of cells
Sample material and quantity:	free lung cells, .x1oE6 macrophages/ gradient
Results expressed in:	-
Equipment:	refractometer: Abbé type, model A, Carl Zeiss, D-7o82 Oberkochen thermostat: type RM 3, Messgeräte-Werk Lauda, Dr. R. Wobser KG, D-697o Lauda-Königshofen gradient fractionator: model 64o, ISCO, Colora Messtechnik GmbH, D-7o73 Lorch 1 centrifuge: OTD-65, rotor: T 865.1, Du Pont Instruments, D-635o Bad Nauheim
Chemicals and reagents:	density gradient medium: colloidal silica sol in cell suspension medium colloidal silica sol: Percoll, no. T 2o21, Deutsche Pharmacia GmbH, D-78oo Freiburg 1 cell suspension medium: MEM, no. 14-33o-54, MEM, Joklik's modification, no. 1F-o68A, Flow Laboratories GmbH, D-53o9 Meckenheim

2501091239

Source: https://www.industrydocuments.ucsf.edu/docs/lghl0000

sucrose, no. 7653,
bromphenol blue, no. 8122,
E. Merck,
D-61oo Darmstadt 1

Procedure

Preparation of gradients:	cells (suspended in colloidal silica sol and cell suspension medium) added to density gradient medium, initial density of mixture adjusted to 1.3436 refractive index (= 1.o66 kg/l buoyant density), total volume: 1o ml/ gradient
Centrifugation:	2.4x1oE5 m/s2 (= 241oo x g) for 3o min at 2o degrees centigrade
Fractionation:	displacement of density gradient by pumping 6oo g/l sucrose solution, containing bromphenol blue, through needle valve into pierced bottom of centrifuge tube. Collection of 12 fractions of approx. o.8 ml volume each

Scientific version: 7.Apr.81
Text version: 23.Sep.81

2501091240

Source: https://www.industrydocuments.ucsf.edu/docs/lghl0000

Differential_cell_counting_(cell_preparation)

Principle:	application of cells to glass slides by centrifugation
Time	
Sampling:	on day ...
Determination:	within ... h after start of harvest of free lung cells
Sample material and quantity:	free lung cells, dependent upon number of cells in the lavage 1oo to 5oo ul
Results expressed in:	-
Equipment:	punched filter paper stripes: SCA oo5, cytocentrifuge: model SCA oo3o, Shandon, Runcorn, Cheshire, United Kingdom slides, no. 9161145, 76 mm x 26 mm, Hirtz und Co., D-5ooo Köln 51
Chemicals and reagents:	-
Procedure:	cells are filled into sample chamber, whose outlet fits into a punched hole of a filter paper strip on top of a glass slide.
Centrifugation:	for 1o min with approx. 3.9x1oE3 m/s2 (= 4oo x g) at RT. Each sample is determined in duplicates.
Scientific version:	22.Aug.79
Text version:	13.Jul.81

2501091241

Source: https://www.industrydocuments.ucsf.edu/docs/lghl0000

Differential cell staining and counting (cytocentrifuge cell sediment)

Principle:	differential staining of cell compartments or components with mixture of acidic, neutral and basic dyes
Time	
Sampling:	on day ...
Determination:	within ... .. after preparation
Sample material and quantity:	free lung cells precipitated on slides with cytocentrifuge
Results expressed in:	(1) 1oE5 macrophages/fraction (2) 1oE5 granulocytes/fraction
Equipment:	microscope: 4oo-fold magnification, standard 14, objective: PL 4o/o.65. Carl Zeiss, D-7o82 Oberkochen cell counter: model MF, no. 7o8238, E. Harig OHG, D-2ooo Hamburg 74 staining trough: glass, no. 9163281, staining tray, no. 91635oo, Hirtz und Co., D-5ooo Köln 51
Chemicals and reagents:	Diff-Quik Stain Set, type Wright -Giemsa, solution A: triaryl methane, 2 mg/l methanole, solution B: 1.25 g/l xanthene in 67 mmol/l phosphate buffer, pH 6.6, solution C: 1.25 g/l thiazine in 67 mmol/l phosphate buffer, pH 6.6, Harleco no. 64851, Merz und Dade AG, CH-3186 Duedingen 2501091242 xylene, no. 8118, Baker Chemicals, D-6o8o Gross-Gerau

Source: https://www.industrydocuments.ucsf.edu/docs/lghl0000

Corbit Balsam,
I. Hecht,
D-23oo Kiel-Hassee

Procedure

Staining: according to Wright-Giemsa

slides are stained:
dipping 5 times in A,
5 times in solution B,
5 times in solution C,
rinsed in distilled water and
finally dried in air

Cell nomenclature: see TABLE ...

Counting and calculation: cells in approx. 5o ocular counting squares (each area (AS) equivalent to 1.44x1oE-2 mm2) counted along 1 diameter of the cell precipitate

precipitate area (AP): 32.2 mm2

calculation:

$$\text{dilution factor (DF)} = \frac{1000}{\text{sample volume}}$$

number of cells/fraction =

$$\text{number of counted cells} \times \frac{AP \times DF}{AS}$$

cells/fraction: (cells/ml) x fraction volume (ml)

Characterization of pulmonary macrophages: during cell counting on stained slides, allocation of pulmonary macrophages to the following categories:

(1) plasma membrane irregularities:
"flowing plasma" or polymorph membrane structure protrusions, blebs, or similar irregularities

2501091243

Source: https://www.industrydocuments.ucsf.edu/docs/lghl0000

(2) polynuclear pulmonary macrophages:

well defined separate nuclei

(3) vacuoles:

number of vacuoles per macrophage, 2o cells determined per selected fraction

vacuole size in pulmonary macrophages, 5 cells determined per selected fraction

(4) size:

cell diameter of pulmonary macrophages, 2o cells determined per selected fracion

Scientific version: 12.Jul.81
Text version: 12.Oct.81

2501091244

Source: https://www.industrydocuments.ucsf.edu/docs/lghl0000

Differential cell staining and counting (cytocentrifuge cell sediment)

Principle:	differential staining of cell compartments or components with mixture of acidic, neutral and basic dyes
Time	
Sampling:	on day ..
Determination:	within ... .. after preparation
Sample material and quantity:	free lung cells precipitated on slides with cytocentrifuge
Results expressed in:	(1) 1oE5 macrophages/fraction (2) 1oE5 granulocytes/fraction
Equipment:	microscope: 4oo-fold magnification, standard 14, objective: PL 4o/o.65 Carl Zeiss, D-7o82 Oberkochen cell counter: model MF, no. 7o8238, E. Harig OHG, D-2ooo Hamburg 74 staining trough: glass, no. 9163281, staining tray, no. 91635oo, Hirtz and Co., D-5ooo Köln 51
Chemicals and reagents:	5oo ml/l ethanolic eosin-methyleneblue (May-Grünwald reagent), no. 1424, 4o ml/l azur-eosin-methyleneblue (Giemsa reagent), no. 92o4, E. Merck, D-61oo Darmstadt 1 xylene, no. 8118, Baker Chemicals, D-6o8o Gross-Gerau Corbit Balsam, I. Hecht, D-23oo Kiel-Hassee

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Procedure

Staining: according to Pappenheim (panoptic stain)

slides are stained:
3 min in eosin-methyleneblue,
18 min in azur-eosin-methyleneblue,
rinsed intensively with distilled water and finally dried in air

Cell nomenclature: see TABLE ..

Counting and calculation: cells in approx. 5o ocular counting squares (each area (AS) equivalent 1.44x1oE-2 mm2) counted along 1 diameter of the cell precipitate

precipitate area (AP): 32.2 mm2

calculation:

$$\text{dilution factor (DF)} = \frac{1000}{\text{sample volume}}$$

$$\text{number of cells/fraction} = \text{number of counted cells} \times \frac{AP \times DF}{AS}$$

cells/fraction: (cells/ml) x fraction volume (ml)

Categories for characterization of pulmonary macrophages: during cell counting on stained slides, allocation of pulmonary macrophages to the following categories (see FIGURE .):

(1) plasma membrane irregularities:
"flowing plasma" or polymorph membrane structure protrusions, blebs, or similar irregularities

(2) polynuclear pulmonary macrophages:
well defined separate nuclei

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Source: https://www.industrydocuments.ucsf.edu/docs/lghl0000

(3) vacuoles:

number of vacuoles per macrophage, 2o cells determined per selected fraction

vacuole size in pulmonary macrophages, 5 cells determined per selected fraction

(4) size:

cell diameter of pulmonary macrophages, 2o cells determined per selected fraction

Scientific version: 12.Jul.81
Text version: 12.Oct.81

2501091247

Source: https://www.industrydocuments.ucsf.edu/docs/lghl0000

Determination of DNA concentration

Principle:	fluorometric determination of the complex between DNA and bisbenzimidazole (Hoechst 33258, (-(2-(hydroxyphenyl)-6-benzimidazolyl)-6-(1-methyl-4-piperanyl)-benzinidazol x 3 HCl)
Time	
Sampling:	on day ...
Determination:	within ... .. after sampling
Sample material and quantity:	(1) homogenate of lavaged lung (2) mouse epidermis extract, approx. 1oo ul
Results expressed in:	(1) ug/g lung WW (2) ug/ml
Equipment:	rotary mixer: model REAX 1R, Heidolph, D-842o Kelheim spectrofluorometer: model 65o-1oS, Perkin Elmer GmbH, D-777o Überlingen
Chemicals and reagents:	sodium dihydrogen phosphate 1-hydrate, no. 6346, disodium hydrogen phosphate 2-hydrate, no. 658o, sodium chloride, no. 64o4, ethylene dinitrilotetraacetic acid disodium 2-hydrate, no. 8418, E. Merck, D-61oo Darmstadt 1 bisbenzimide (Hoechst 33258), no. 33217, Riedel de Haen AG, D-3o16 Seelze calf thymus DNA, no. 1o4167, Boehringer Mannheim GmbH, D-68oo Mannheim 31

2501091248

Source: https://www.industrydocuments.ucsf.edu/docs/lghl0000

final concentrations of components in assay mixture:

NaH_2PO_4/Na_2HPO_4	5o mmol/l
NaCl	2 mol/l
EDTA	2 mmol/l
bisbenzimidazole (Hoechst 33258)	1 mg/l

Procedure

Extraction: will be given in the final report

Determination: according to Zabarca, C. and Paigen, K., Anal. Biochem. 1o2: 344-352 (198o), and to Cesarone, C.F. et al., Anal. Biochem. 1oo: 188-197 (1979)

fluorometric determination:

excitation wavelength: 356 nm
emission wavelength : 458 nm

standard curve: see FIGURE ...

Scientific version: 6.Feb.81
Text version: 9.Oct.81

2501091249

Source: https://www.industrydocuments.ucsf.edu/docs/lghl0000

Determination of DNA

Principle:	photometric determination of the colored complex between diphenylamine and the deoxypentose
Time	
Sampling:	on day ...
Determination:	within ... .. after sampling
Sample material and quantity:	purified nucleic acid fraction, approx. 2o ul
Results expressed in:	ug
Equipment:	spectrophotometer: model 555, Perkin Elmer GmbH, D-777o Überlingen
Chemicals and reagents:	aqueous acetaldehyde diphenylamine reagent: acetaldehyde, no. ooo7o, Fluka Feinchemikalien GmbH, D-791o Neu-Ulm diphenylamine, no. 3o86, E. Merck, D-61oo Darmstadt 1 calf thymus DNA, no. 1o4167, Boehringer Manheim GmbH, D-68oo Mannheim 31
Procedure:	according to Burton, K., in: Grossman, L., Maldare, K., (Eds.): Methods in Enzymology, Vol. 12, part B, New York-London: Acad. Press, 1968, pp. 163-166 standard curve: see FIGURE ...
Scientific version:	17.Mar.8o
Text version:	12.Jul.81

2501091250

Source: https://www.industrydocuments.ucsf.edu/docs/lghl0000

Determination of dry weight

Principle:	gravimetric determination of freeze-dried sample
Time	
Sampling:	-
Determination:	-
Sample material and quantity:	rat lung, 5/group
Results expressed in:	mg
Equipment:	freeze drier: model GT 3, Leybold-Heraeus GmbH, D-5ooo Köln 51 tubes: no. 2o7o, conical graduated, polypropylene, diameter: 3o mm, length: 115 mm, Falcon, Div. Becton and Dickinson GmbH, D-69oo Heidelberg-Wieblingen analytical balance: no. 2oo1 MP2, Sartorius GmbH, D-34oo Göttingen
Chemicals and reagents:	liquid nitrogen
Procedure:	sample of known fresh weight frozen in liquid nitrogen and freeze-dried until vacuum in drying chamber remained constant when the vacuum pump of the freeze drier was shut off (time: approx. 5o h). weigthing of dried sample
Scientific version:	21.Sep.8o
Text version:	28.Sep.81

2501091251

Source: https://www.industrydocuments.ucsf.edu/docs/lghl0000

Preparation, homogenization and centrifugation of epidermis

Principle:	epidermal sheets removed mechanically from dermis. Mechanical homogenization of sheets and removal of cell debris and mitochondria by centrifugation
Time	
Sampling:	on day ...
Determination:	.. h after last application
Sample material and quantity:	application skin areal, skin of .. mice pooled
Results expressed in:	-
Equipment:	homogenizer: Ultra-turrax 18/1o, Janke und Kunkel, D-7813 Staufen centrifuges: RC-5B, rotor: SS-34, OTD-65, rotor: T 865.1 Du Pont Instruments, D-635o Bad Nauheim test tubes: no. o3113, polycarbonate, Du Pont Instruments, D-635o Bad Nauheim
Chemicals and reagents:	depilatory cream: Pilca, Olivin, D-2ooo Hamburg homogenization buffer: sodium phosphate, 5o mmol/l, pH 7.2, containing o.1 mmol/l pyridoxal phosphate, 1 mmol/l 1.4-dithio-erythrit and o.1 mmol/l EDTA, sodium dihydrogen phosphate, no. 6346, disodium hydrogen phosphate, no. 658o, ethylenediaminetetraacetic acid (EDTA), no. 8418, E. Merck, D-61oo Darmstadt 1

250109125

Source: https://www.industrydocuments.ucsf.edu/docs/lghl0000

pyridoxal-5-phosphate, no. P 9255,
Sigma Chemie GmbH,
D-8o21 Taufkirchen

1,4-dithioerythrit, no. 223662,
Boehringer Mannheim GmbH,
D-68oo Mannheim 31

Procedure

Separation of epidermis from dermis:	removed skin areal placed on surface of aluminium block (approx. 4 degrees centigrade), scraping of epidermal surface with scalpel, removed sheets immediately added to homogenization buffer (4 degrees centigrade)
Homogenization:	mechanical grinding of epidermal sheets at approx. 4 degrees centigrade, o.5 ml homogenization buffer per mouse, pH 7.2
Centrifugation:	homogenate: 9.8x1oE4 m/s2 (= 1oooo x g) for 1o min at 4 degrees centigrade supernatant: 9.8x1oE5 m/s2 (= 1ooooo x g) for 6o min at 4 degrees centigrade (supernatant: postmicrosomal)
Storage:	up to 1 month at minus 8o degrees centigrade

Scientific version: 1.Feb.81
Text version: 22.Jul.81

2501091253

Source: https://www.industrydocuments.ucsf.edu/docs/lghl0000

Determination of erythrocyte glycolytic activity

Principle:	incubation of ery. suspended in glucose containing perfusion medium in shaking water bath, determination of glucose concentration in the supernatant at the beginning and at the end of the incubation
Time	
Sampling:	-
Determination:	-
Sample material and quantity:	rats no. ..., approx. 2o ml perfusate
Results expressed in:	(1) glucose uptake: umol/min (2) lactate efflux: umol/min
Equipment:	shaking water bath, no. 3o47, Köttermann, D-3165 Hänigsen plastic incubation tubes with screw caps, no. 2o7o, Falcon, Div. Becton and Dickinson GmbH, D-69oo Heidelberg-Wieblingen
Chemicals and reagents:	-
Procedure:	perfusate with ery. added to incubation tube, tube closed and shaken in waterbath at 37 degrees centigrade for 12o min
Scientific Version:	21.Jul.8o
Text version:	23.Sep.81

2501091254

Source: https://www.industrydocuments.ucsf.edu/docs/lghl0000

Preparation of erythrocytes

Principle:	centrifugation and repetitive washing with physiological saline
Time	
Sampling:	-
Determination:	daily within ... hours before start of perfusion
Sample material and quantity:	citrate blood, rat, variable quantity
Results expressed in:	-
Equipment:	centrifuge: model J6, rotor: JS-4.2, Beckman Instruments GmbH, D-8000 München 40 plastic tubes: no. 2070, Falcon, Div. Becton and Dickinson GmbH, D-6900 Heidelberg-Wieblingen
Chemicals and reagents:	magnesium sulfate 7-hydrate, no. 5886, potassium chloride, no. 4936, calcium chloride 2-hydrate, no. 2382, citric acid 1-hydrate, no. 244, trisodium citrate 2-hydrate, no. 6447, D(+)-glucose 1-hydrate, no. 8346, potassium dihydrogen phosphate, no. 4873, sodium chloride, no. 6404, diethyl ether, no. 923, E. Merck, D-6100 Darmstadt 1 bovine serum albumin (BSA), no. A 4503, Sigma Chemie GmbH, D-8021 Taufkirchen

2501091255

Source: https://www.industrydocuments.ucsf.edu/docs/lghl0000

citrate solution:
2o.5 g/l glucose,
7.87 g/l trisodium citrate,
o.55 g/l citric acid,
4.16 g/l sodium chloride

erythrocyte suspension medium:
Krebs-Henseleit solution:
6.9o g/l sodium chloride,
o.35 g/l potassium chloride,
o.37 g/l calcium chloride 2-hydrate,
o.29 g/l magnesium sulfate 7-hydrate,
o.16 g/l potassium dihydrogen phosphate,
2.1o g/l sodium hydrogen carbonate,
1.1o g/l glucose

Procedure

Collection: rats treated with lethal dose of diethyl ether, the heart punctured in the right ventricle and the collected blood immediately diluted 1 + 1 (1/1) with citrate solution, 4 degrees centigrade

Washing: ery. sedimented by centrifugation with 9.8x1oE3 m/s2 (= 1ooo x g) at 1o degrees centigrade. Supernatant and white layer on top of ery. pellet (leucocytes etc.) aspirated and discarded. After 2 washings with 9 g/l sodium chloride and 5 g/l bovine serum albumin, ery. resuspended in Krebs-Henseleit solution, pH 7.6

Scientific version: 5.Feb.8o
Text version: 16.Jul.81

2501091256

Source: https://www.industrydocuments.ucsf.edu/docs/lghl0000

Evaluation of stained proteins in polyacrylamide gels

Principle:	photometric determination and determination of peak height and migration distance from digitalized gel scan
Time:	-
Sample material and quantity:	Coomassie brilliant blue R25o stained (slabs or cylindrical) polyacrylamide gels
Results expressed in:	(1) migration distance (cm) (2) peak area (arbitrary units) (3) relative peak area (o/o)
Equipment:	Dual wavelength scanner: CS91o, Shimadzu Europe, D-4ooo Düsseldorf recorder: 221o, LKB Instruments, D-8o32 Gräfelfing Orthoplex TM Coordinate Sensor: no. 3825-1, Elographics Inc., Oak Ridge, Tennessie, USA Teletype ASR 33, Vollwood, D-4ooo Düsseldorf
Chemicals and reagents:	acetic acid, no. 62E, propanol-2, no. 9634, E. Merck, D-61oo Darmstadt 1

2501091257

Source: https://www.industrydocuments.ucsf.edu/docs/lghl0000

Procedure

Photometric scanning:	stained gels positioned in the beam of scanning photometer, beam focused in the middle of gel slot sample wavelength: 4oo nm reference wavelength: 56o nm mode: absorbance, slit size: 1.7 mm x o.15 mm, scan speed: 2o mm/min
Recording:	variable mode, 1o to 5oo mV according to intensity of bands slot and scanned from top to bottom of gel
Evaluation:	assignment of identical numbers for peaks and/or regions of gels to be compared, similar base line for the 2 gels drawn to interpolate the local minimum for each peak and the height based on this point

Scientific version: 7.Jan.81
Text version: 25.Jun.81

2501091258

Source: https://www.industrydocuments.ucsf.edu/docs/lghl0000

Extraction and hydrolization of rat lung

Principle:	extraction of phospholipids in chloroform, methanol. Hydrolization of extract with perchlor acid
Time	
Sampling:	on days ...
Determination:	within ... .. after sampling
Sample material and quantity:	lung homogenate, 2 ml
Results expressed in:	-
Equipment:	homogenizer: Potter-Elvehjem, glass, with motor driven teflon pestle, Braun Melsungen AG, D-35o8 Melsungen centrifuge: model J6, rotor: JS-4.2, Beckmann Instruments GmbH, D-8ooo München 4o metal block thermostat: Gebr. Liebisch, D-48oo Bielefeld 14 glass ware, Faust GmbH, D-5ooo Köln 9o
Chemicals and reagents:	phosphate buffered saline (PBS), Instamed PBS, calcium- and magnesium free, no. 182-1, pH 7.2, Seromed GmbH, D-8ooo München 71 methanol, no. 8o45, Baker Chemicals, D-6o8o Gross-Gerau

2501091259

Source: https://www.industrydocuments.ucsf.edu/docs/lghl0000

chloroform, no. 2445,
sodium chloride, no. 64o4,
perchloric acid (PCA), no. 519,
E. Merck,
D-61oo Darmstadt 1

Procedure: according to Folch, J., Lees, M. and Sloane-Stanley, G.H., J. Biol. Chem. 226: 497 (1957)

Homogenization, Extraction: in chloroform/methanol/NaCl mixture

Centrifugation: 9.8x1oE3 m/s2 (= 1ooo x g = 22oo rpm) for 15 min at 4 degrees centigrade

lower phase semared and dried with nitrogen at 5o degrees centigrade

Hydrolization: with PCA at 2oo degrees centigrade for approx. 1 to 2 h. Hydrolysate diluted 1 plus 1 with bidistilled water

before use glass ware must be clean with chromosulfuric acid

Scientific version: 22.Sep.81
Text version: 22.Sep.81

2501091260

Source: https://www.industrydocuments.ucsf.edu/docs/lghl0000

Determination of flow rate

Principle:	volumetric determination
Time:	sampling .. min after start of perfusion
Sample material and quantity:	perfusate, 5 to 1o ml
Results expressed in:	ml/min
Equipment:	graduated cylinder, 1o ml, stop watch
Chemicals and reagents:	-
Procedure:	sampling of the perfusate for 3o or 6o s and determination of the volume in graduated cylinder
Scientific version:	3.Dec.79
Text version:	24.Sep.81

2501091261

Source: https://www.industrydocuments.ucsf.edu/docs/lghl0000

Gas chromatographic determination of nicotine and cotinine in plasma

Principle:	gas chromatographic determination after sequential extraction of nicotine and cotinine from plasma
Time:	-
Sample material and quantity:	plasma, 4oo ul
Results expressed in:	ug/l
Equipment:	gas chromatograph: HP 584oA detector: AFID, Hewlett-Packard GmbH, D-6ooo Frankfurt/Main
Chemicals and reagents:	nicotine, no. 77635, Serva Feinbiochemica GmbH und Co., D-69oo Heidelberg cotinine, 1 ml aqueous solution (5oo mg/ml), Labor Dr. R. Hallermayer, D-89oo Augsburg lidocaine hydrochloride, Sonnen Apotheke, D-5ooo Köln 9o quinoline, no. 8o24o7, diisopropyl ether, no. 867, dichloromethane, no. 822271, acetone, no. 14, sodium hydroxide (2oo g/l), no. 5594, hydrochloric acid (1 mol/l), no. 9o57, E. Merck, D-61oo Darmstadt 1 helium, hydrogen, air (synthetic), Linde AG, D-5ooo Köln 5o

2501091262

Source: https://www.industrydocuments.ucsf.edu/docs/lghl0000

Procedure

Extraction:	addition of 5o ul of the internal standard solution (5oo ng quinoline/ml o.1 mol/l hydrochloric acid), 2oo ul sodium hydroxide (2oo g/l) and 1 ml diisopropyl ether to 4oo ul plasma. After agitation (5 min) and centrifugation (approx. 7.8x1oE3 m/2 = 8oo x g, 5 min, 1o degrees centigrade) transfer to the ether phase to a second centrifuge tube and reextraction with 1oo ul hydrochloric acid (1 mol/l). Evaporation of ether phase, alkalization of aqueous residue with 1oo ul sodium hydroxide (2oo g/l), extraction with 1oo ul diisopropyl ether and injection of an ether phase aliquot into the gas chromatograph
	for extraction of cotinine, addition of 5o ul internal standard solution (2 ug lidocaine hydrochloride/ml water) and 1 ml dichloromethane to plasma remaining after extraction of nicotine. After agitation and centrifugation removing of water phase, evaporation of dichloromethane, dissolution of residue with 5o ul acetone and injection of an aliquot into the gas chromatograph

Gas chromatography

Determination of nicotine

Column:	2 m x 3 mm inner diameter, glass
Column packing:	3 o/o SP-225o DB on Supelcoport, 1oo to 12o mesh
Carrier gas and flow rate:	helium, 3o ml/min
Oven temperature:	15o degrees centigrade
Injection port temperature:	2oo degrees centigrade
Detector temperature:	3oo degrees centigrade

2501091263

Source: https://www.industrydocuments.ucsf.edu/docs/lghl0000

Determination of cotinine

Column:	1.2 m x 2 mm inner diameter, glass
Column packing:	2 o/o FFAP on Chromosorb W-HP, 8o to 1oo mesh
Carrier gas and flow rate:	helium, 3o ml/min
Oven temperature:	175 degrees centigrade
Injection port temperature:	2oo degrees centigrade
Detector temperature:	3oo degrees centigrade

Computation:	addition of different amounts of nicotine and cotinine to plasma, extraction and chromatography as described above and construction of calibration curves for nicotine and cotinine with peak heights as function of concentration
Scientific version:	7.Jan.81
Text version:	9.Oct.81

2501091264

Source: https://www.industrydocuments.ucsf.edu/docs/lghl0000

Determination of glucose concentration

Principle:	photometric determination of NADH produced during oxidation of glucose to gluconolactone (glucose dehydrogenase, (EC 1.1.1.47))
Time	
Sampling:	-
Determination:	within ... .. after sampling
Sample material and quantity:	(1) serum, 5 ul (2) perfusate supernatant, 5 ul (3) muscle tissue extract, deproteinized
Results expressed in:	(1) mmol/l (2) rate of consumption: umol/(min x g muscle WW)
Equipment:	ABA-1oo Bichromatic Analyzer, Abbott Laboratories, Diagnostics Division, So. Pasadena, California 91o3o, USA
Chemicals and reagents:	Merckotest Glucose, no. 3389, E. Merck, D-61oo Darmstadt 1 standard: Precipath S, no. 125199, Boehringer Mannheim GmbH, D-68oo Mannheim 31
Procedure:	according to Banauch, D., Bruemmer, W., Ebeling, W., Metz, H., Rindfrey, H., Lang, H., Leybold, K., and Rick, W., Z. Klin. Chem. Klin. Biochem. 13: 1o1-1o7 (1975) photometric determination: wavelength 1: 34o nm wavelength 2: 38o nm

2501091265

Source: https://www.industrydocuments.ucsf.edu/docs/lghl0000

storage until determination:
4 degrees centigrade

calculation of glucose consumption:
calculation of difference of the glucose concentration determined at various times after start of perfusion

correction of consumption for
(1) continuous glucose addition
(2) loss of perfusate (and glucose) due to sampling and leakage (a)

Scientific version: 11.Sep.78
Text version: 25.Jun.81

2501091266

Source: https://www.industrydocuments.ucsf.edu/docs/lghl0000

Determination of glycogen concentration

Principle:	photometric determination of NADPH formed during the oxidation of glucose-6-phosphate. Glucose-6-phosphate is obtained by phosphorylation of glucose (hexokinase, EC 2.7.1.1), which is hydrolyzed enzymatically from glycogen by amyloglucosidase (EC 3.2.1.3).
Time	
Sampling:	-
Determination:	-
Sample material and quantity:	muscle tissue extract, deproteinized
Results expressed in:	mg/kg WW
Equipment:	spectrophotometer: model 555, Perkin Elmer GmbH, D-777o Überlingen shaker water bath: no. 3o47, Köttermann, D-3165 Hänigsen
Chemicals and reagents:	triethanolamine hydrochloride, no. T 15o2, Sigma Chemie GmbH, D-8o21 Taufkirchen ATP, no. 126888, NADP, no. 1o7727, glucose-6-phosphate dehydrogenase (EC 1.1.49), no. 127o43, hexokinase (EC 2.7.1.1), no. 127175, Boehringer Mannheim GmbH, D-68oo Mannheim 31 magnesium chloride, no. 5886, E. Merck, D-61oo Darmstadt 1

2501091267

Source: https://www.industrydocuments.ucsf.edu/docs/lghl0000

Procedure:	according to Keppler, D., Decker, K., in: Methoden der enzymatischen Analyse, Vol. 2, Verlag Chemie Weinheim, 3rd ed., 1974, pp. 1171-1176
	incubation: in shaker water bath for 2 h, 4o degrees centigrade
	photometric determination:
	wavelength: 334 nm
Scientific version:	16.Apr.8o
Text version:	23.Sep.81

2501091268

Source: https://www.industrydocuments.ucsf.edu/docs/lghl0000

Determination of glyoxylate concentration

Principle:	photometric determination of NADH oxidation during the reduction of glyoxylate to glycolate catalyzed by spinach glyoxylate reductase (EC 1.1.1.26)
Time	
Sampling:	-
Determination:	-
Sample material and quantity:	(1) perfusate, o.5 ml (2) muscle tissue extract, deproteinized
Results expressed in:	(1) mg/l (2) mg/g WW
Equipment:	spectrophotometer: model 555, Perkin Elmer GmbH, D-777o Überlingen
Chemicals and reagents:	mono-potassium dihydrogen-phosphate, no. 4873, di-potassium hydrogen-phosphate, no. 51o4, sodium bicarbonate, no. 6329E, E. Merck, D-61oo Darmstadt 1 NADH, no. 1o7727, glyoxylate reductase (= glycolate dehydrogenase, EC 1.1.1.26) from spinacea oleracea, 55 U/mg, no. 1o6151, Boehringer Mannheim GmbH, D-68oo Mannheim 31
Procedure:	according to Bergmeyer, H.U. and Gutmann, J., in: Bergmeyer, H.U. (ed.): Methoden der enzymatischen Analyse, Vol. 2, Verlag Chemie Weinheim, 3rd edition, 1974, pp. 1563-1565

2501091269

Source: https://www.industrydocuments.ucsf.edu/docs/lghl0000

photometric determination:

wavelenght: 34o nm

standard curve: see FIGURE ..

Scientific version: 1o.Apr.8o
Text version: 23.Sep.81

2501091270

Source: https://www.industrydocuments.ucsf.edu/docs/lghl0000

Harvest of free lung cells from rat lung by lavage

Principle:	pulmonary lavage through cannulated trachea
Time	
Sampling:	on day ...
Determination:	-
Sample material and quantity:	albino rats, .. rats/group
Results expressed in:	-
Equipment:	cannula: stainless steel (V2A), outer diameter: 1 mm, length: 45 mm, sterile, Faust GmbH, D-5ooo Köln 9o cannula: sterile, no. 941o118, syringes: 1o ml, sterile, no. 941oo1o, 2 ml, sterile, no. 941ooo2, Hirtz und Co., D-5ooo Köln 51 test tubes: no. 2o7o, conical graduated, polypropylene, diameter: 3o mm, length: 115 mm, Falcon, Div. Becton and Dickinson GmbH, D-69oo Heidelberg-Wieblingen
Chemicals and reagents:	phosphate-buffered saline (PBS), Instamed PBS, calcium-and magnesium-free, no. 182-1, pH 7.2, Seromed GmbH, D-8ooo München 71 pentobarbital (Nembutal), Abbott GmbH, D-236o Bad Segeberg

2501091271

Source: https://www.industrydocuments.ucsf.edu/docs/lghl0000

Procedure

Sacrifice:	rats killed with letal dose of pentobarbital (3o mg/kg BW)
Lavage:	free lung cells removed from the tracheobronchial and alveolar surface by 1o times instillation and subsequent withdrawal of the same PBS lavage solution (8 ml/rat) through the cannulated trachea, storage on ice

Scientific version: 23.Sep.8o
Text version: 12.Jul.81

2501091272

Source: https://www.industrydocuments.ucsf.edu/docs/lghl0000

Harvest of spleens

Principle:	spleens from killed mice harvested under aseptical conditions and squeezed in order to form single cell suspensions
Time:	-
Sample material and quantity:	immunized BALB/c mice
Results expressed in:	-
Equipment:	forceps, scissors, scalpel, petri dishes: 9 cm diameter,TC quality, Falcon, Div. Becton and Dickinson GmbH, D-69oo Heidelberg-Wieblingen
Chemicals and reagents:	ethanol, 7oo ml/l, no. 972, E. Merck, D-61oo Darmstadt 1 growth medium: RPMI 164o, Seromed GmbH, D-8ooo München 71
Procedure:	mice killed by cervical dislocation, fixed on a preparation plate and rinsed with 7oo ml/l ethanol abdomen opened by use of forceps and scissors under aseptic conditions, removal of spleen, spleen placed in a petri dish containing 1o ml of growth medium. Spleen opened at the two ends and squeezed out by use of forceps resulting in a single cell suspension
Scientific version: Text version:	Oct.8o 24.Sep.81

2501091273

Source: https://www.industrydocuments.ucsf.edu/docs/lghl0000

Determination of hemoglobin concentration

Principle:	photometric determination
Time	
Sampling:	-
Determination:	within 6o min after preparation of perfusate
Sample material and quantity:	perfusate, 4o ul
Results expressed in:	mmol/l (Mr: 16144)
Equipment:	Coulter Hemoglobinometer, Coulter Electronics Limited, D-415o Krefeld
Chemicals and reagents:	electrolyte: Isoton 2 with Zapoglobin, Coulter Electronics Limited, D-415o Krefeld
Procedure:	acc. to manufacturer's manual
Scientific version:	3o.Jan.8o
Text version:	19.Aug.81

2501091274

Source: https://www.industrydocuments.ucsf.edu/docs/lghl0000

Determination of hemoglobin concentration

Principle:	photometric determination of hemiglobincyanid hemoglobin + cyanide + ferricyanide --> hemiglobincyanide
Time	
Sampling:	-
Determination:	-
Sample material and quantity:	perfusate, 2o ul
Results expressed in:	g/l
Equipment:	spectrophotometer: model 555, Perkin Elmer GmbH, D-777o Überlingen
Chemicals and reagents:	test set Hämoglobin, no. 124729, Boehringer Mannheim GmbH, D-68oo Mannheim 31
Procedure:	acc. to van Kampen, E.J. and Zijlstra, W.G. in Clin. Chim. Acta 6: 538 (1961) photometric determination: wavelength: 546 nm
Scientific version: Text version:	22.Sep.8o 23.Sep.81

2501091275

Source: https://www.industrydocuments.ucsf.edu/docs/lghl0000

Determination of hemolysis

Principle:	subjective optical examination of perfusate supernatants (a)
Time	
Sampling:	-
Determination:	-
Sample material and quantity:	total perfusate supernatant per sample
Results expressed in:	arbitrary units: o: no hemolysis 1: weak hemolysis 2: medium hemolysis 3: strong hemolysis
Equipment:	-
Chemicals and reagents:	-
Procedure:	-
Scientific version:	17.Jul.8o
Text version:	23.Sep.81

2501091276

(a) detection limit: o.2 g/l hemoglobin (Behrendt, H., Chemistry of erythrocytes, Thomas, Springfield 1957)

Source: https://www.industrydocuments.ucsf.edu/docs/lghl0000

Determination of hemolysis

Principle:	spectrophotometric determination of hemoglobin absorption
Time	
Sampling:	-
Determination:	within ... hours after preparation of perfusate supernatant
Sample material and quantity:	perfusate supernatant, 5o ul dilution 1 : 2o with perfusion medium without erythrocytes
Results expressed in:	umol/l
Equipment:	spectrophotometer: model 555, Perkin Elmer GmbH, D-777o Überlingen
Chemicals and reagents:	-
Procedure	
Photometric determination:	wavelength of zero setting: 49o nm wavelength for determination: 415 nm (see FIGURE .) blank: perfusion medium without erythrocytes
Calculation:	from standard curve, obtained with defined hemoglobin concentrations typical standard curve: see FIGURE ...
Scientific version:	8.Jan.81
Text version:	23.Sep.81

2501091277

Source: https://www.industrydocuments.ucsf.edu/docs/lghl0000

Determination of hematocrit

Principle:	centrifugation
Time	
Sampling:	-
Determination:	-
Sample material and quantity:	perfusate, 75 ul
Results expressed in:	o/o
Equipment:	heparinized capillary tubes, no. A8o3, Gelman-Hawksley Ltd., Lancing, Sussex, United Kingdom centrifuge: microrapid, Hettich GmbH, D-72oo Tuttlingen sealing wax, D 558, Radiometer A/S, DK-24oo Copenhagen, Denmark
Chemicals and reagents:	-
Procedure:	capillary tube dipped into perfusate until defined volume of tube is filled with sample, tube closed on either side and then inserted into centrifuge rotor
Centrifugation:	approx. 5.4x1oE4 m/s2 (= 55oo x g), 1o min, RT reading of ery. column length
Scientific version:	5.Feb.8o
Text version:	23.Sep.81

2501091278

Source: https://www.industrydocuments.ucsf.edu/docs/lghl0000

Hematocrit (Particle counter method)

Principle:	counting of erythrocytes with particle counter, calculation with hematocrit unit
Time	
Sampling:	-
Determination:	within 6o min after preparation of perfusate
Sample material and quantity:	perfusate, 4o ul
Results expressed in:	o/o
Equipment:	Coulter Counter, Coulter Hct.-option, Coulter Electronics Limited, D-415o Krefeld
Chemicals and reagents:	-
Procedure:	according to manufacturer's manual
Scientific version:	23.Jul.8o
Text version:	27.Feb.81

2501091279

Source: https://www.industrydocuments.ucsf.edu/docs/lghl0000

High Pressure Liquid Chromatography

Principle:	isocratic chromatographic separation based on differential liquid-solid partition of substances between organic nonpolar phase chemically bonded to solid silica particles and polar liquid-mobile phase (reversed phase principle) at high-pressure
Time:	within 24 h after preparation of test or reference substance solution
Sample material and quantity:	test or reference substances dissolved in bidistilled water, 2o ul equiv. to o.o68 to o.2o3 umol/injection
Results expressed in:	relative units (peak area)
Equipment:	high-pressure liquid chromatograph: model 1o84 B, detector: variable wavelength UV-detector 1o3o B, Hewlett Packard, D-75oo Karlsruhe column: RP-18, 25o mm x 4.6 mm, Dr. H. Knauer Wissenschaftliche Geräte KG, D-637o Oberursel injection vial: no. 8o184, 11.5 mm x 325 mm, Müller und Müller Glaswarenfabrik, D-345o Holzminden analytical balance: model 2oo1 MP2, Sartorius GmbH, D-34oo Göttingen
Chemicals and reagents:	phosphoric acid: 1 l = 1.71 kg, no. 6o24, Baker Chemikalien, D-6o8o Gross-Gerau

2501091280

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citric acid, no. 244,
oxalic acid, no. 495,
E. Merck,
D-61oo Darmstadt 1

L-lactic acid, no. L 175o,
Sigma Chemie GmbH,
D-8o28 Taufkirchen

Procedure

HPLC separation

Injection volume:	2o ul
Column:	RP-18, particle diameter: 1o um, column length: 25o mm, inner diameter: 4.6 mm
Oven and solvent temperature:	RT
Mobile phase:	bidistilled water titrated with phosphoric acid to pH 2.3
Flow rate:	o.8 ml/min
Column pressure:	31 to 39 bar
Detection:	UV-absorbance, wavelength: 2oo nm

Calculations: standard curve for determination of glyoxylic acid: see FIGURE ..

Scientific version: 23.Feb.81
Text version: 23.Jul.81

2501091281

Source: https://www.industrydocuments.ucsf.edu/docs/lghl0000

Homogenization, extraction and hydrolization of free lung cells

Principle:	homogenization of free lung cells and extraction of phospholipids in chloroform, methanol. Hydrolization of extract with perchlor acid
Time	
Sampling:	on days ...
Determination:	within ... .. after sampling
Sample material and quantity:	free lung cells, 1oE5 to 1oE6 cells per determination
Results expressed in:	-
Equipment:	homogenizer: Potter-Elvehjem, glass, with motor driven teflon pestle, Braun Melsungen AG, D-35o8 Melsungen centrifuge: model J6, rotor: JS-4.2, Beckmann Instruments GmbH, D-8ooo München 4o metal block thermostat: Gebr. Liebisch, D-48oo Bielefeld 14 glass ware: Faust GmbH, D-5ooo Köln 9o
Chemicals and reagents:	methanol, no. 8o45, Baker Chemicals, D-6o8o Gross-Gerau chloroform, no. 2445, sodium chloride, no. 64o4, perchloric acid (PCA), no. 519, E. Merck, D-61oo Darmstadt 1

2501091282

Source: https://www.industrydocuments.ucsf.edu/docs/lghl0000

Procedure:	according to Folch, J., Lees, M. and Sloane-Stanley, G.H., J. Biol. Chem. 226: 497 (1957)
Homogenization, Extraction:	in chloroform/methanol/NaCl mixture
Centrifugation:	9.8x1oE3 m/s2 (= 1ooo x g) for 15 min at 4 degrees centigrade
	lower phase semared and dried with nitrogen at 5o degrees centigrade
Hydrolization:	with PCA at 2oo degrees centigrade for approx. 1 to 2 h. Hydrolysate diluted 1 plus 1 with bidistilled water
	before use glass ware must be clean with chromosulfuric acid
Scientific version:	22.Sep.81
Text version:	24.Sep.81

2501091283

Source: https://www.industrydocuments.ucsf.edu/docs/lghl0000

Determination of hydrogen-ion concentration

Principle:	electrochemical determination
Time:	on day of preparation
Sample material and quantity:	(1) perfusate, approx. o.2 ml (2) assay buffer, approx. o.5 ml
Results expressed in:	pH (negative decadic logarithm of hydrogen ion concentration
Equipment:	Astrup Micro Equipment: AME1 or BMS3 MK2 and Digital Acid-Base Analyzer PHM7 MK2, Radiometer A/S, DK-24oo Copenhagen, Denmark via Hillerkus D-415o Krefeld-Bochum
Chemicals and reagents:	calibration buffers: no. S 15oo (pH 6.841) and no. S 151o (pH 7.383), Radiometer A/S, DK-24oo Copenhagen, Denmark
Procedure:	determination of pH with a calibrated glass electrode (2 calibration points) at 38 degrees centigrade
Scientific version: Text version:	5.Feb.8o 25.Jun.81

2501091284

Source: https://www.industrydocuments.ucsf.edu/docs/lghl0000

Determination of hydrogen-ion concentration

Principle:	electrochemical determination
Time:	on day of preparation
Sample material and quantity:	(1) lavage media, approx. 2.5 ml (2) cell suspensions, approx. 2.5 ml (3) assay buffer, approx. 2.5 ml (4) application stock solution, 2 ml (5) perfusate, o.2 ml
Results expressed in:	pH (= negative decadic logarithm of hydrogen ion concentration)
Equipment:	pH meter: PW 94o9, glass electrode: CA/14o2, Philips GmbH, D-35oo Kassel
Chemicals and reagents:	calibration buffers: pH 7.oo, no. 9887, pH 6.oo, no. 9886, pH 4.oo, no. 9884, E. Merck, D-61oo Darmstadt 1
Procedure:	determination at RT after calibration of pH meter with 2 standard buffers
Scientific version: Text version:	28.Feb.79 8.Oct.81

2501091285

Source: https://www.industrydocuments.ucsf.edu/docs/lghl0000

Immunization

Principle:	rise of antibodies against the nicotine and cotinine immunogens administered together with complete Freund' adjuvant to rabbits and mice, booster injections together with incomplete Freund' adjuvant, mice immunologically activated by injection of bacterial lipopolysaccharide 1 day after immunogen injection
Time:	-
Sample material and quantity:	rabbits: specific amounts of immunogen for injection per treatment see below, 2o to 3o intradermal injections mice: specific amounts of immunogen for injection per treatment see below, i. p. injections
Equipment:	sonicator B15 with titanium micro-tip, Branson Sonic Power Company, Danbury, Connecticut, USA
Chemicals and reagents:	bacto adjuvant, complete Freund, no. o66o-5721, bacto adjuvant, incomplete Freund, no. 66o-572o, phosphate-buffered saline (PBS), no. 4o8, Gibco Biocult, D-75oo Karlsruhe 21 bacterial lipopolysaccharide B from Salmonella Typhosa o9o1, no. 3946-1o, Difco Laboratories, Detroit Michigan, USA, via O. Nordwald KG, D-2ooo Hamburg 5o

2501091286

Source: https://www.industrydocuments.ucsf.edu/docs/lghl0000

Procedure

Immunogen preparation:

(1) for priming (= 1st injection):

immunogen (2oo ug/rabbit, 1oo ug/mouse) dissolved in PBS (o.5 ml/rabbit, o.25 ml/mouse) and emulsified with complete Freund' adjuvant (CFA, 1.5 ml/rabbit, o.75 ml/mouse, Mishell et al., 198o) by ultrasonication under semisterile conditions

(2) for booster injection (2nd and subsequent injections):

immunogen (1oo ug/rabbit, 2o ug/mouse) dissolved in o.25 ml PBS, emulsified with o.75 ml of incomplete Freund' adjuvant (IFA) as described and emulsions used within 1 h after preparation

(3) for final injection (mice): non emulsified immunogen solution

Rabbit immunization (a):

(1) priming: hair on the back and on the proximal parts of all 4 limbs sheared, o.o5 ml immunogen priming emulsion intradermal administered at 24 sites along the back and at 4 sites at each limb with tuberculin syringe and fine needle

(2) booster injection: i. m. injections, starting 1o weeks after priming: o.5 ml of the immunogen booster emulsion into each hind limb. Depending on the antibody titer, further injections after minimum intervals of 4 weeks

Mouse immunization (a, b):

(1) priming: immunogen priming emulsion (CFA) injected i. p.

(a) according to Hurn and Chantler (198o)
(b) according to Stähli et al. (198o)

2501091287

Source: https://www.industrydocuments.ucsf.edu/docs/lghl0000

24 h later: 1o ug of bacterial lipopolysaccharide (LPS) (a) dissolved in o.5 ml PBS, injected i. p.

(2) booster injection: 3 weeks (minimum 2 weeks) after priming half the above dose of immunogen emulsified with IFA injected i. p.

24 h later: 1o ug of bacterial lipopolysaccharide (LPS) (a) dissolved in o.5 ml PBS, injected i. p.

(3) final injection: on the 3 days prior to spleen harvest 1 injection/d of half the priming dose of soluble, not emulsified immunogen

Scientific version: Oct.8o
Text version: 23.Sep.81

(a) according to Lemke et al. (1979)
(b) according to Lemke et al. (1979)

2501091288

Source: https://www.industrydocuments.ucsf.edu/docs/lghl0000

Determination of inorganic phosphate (malachit green method)

Principle:	photometric determination of a phosphomolybdate-malichite green complex at 66o nm
Time	
Sampling:	on days ...
Determination:	within ... .. after sampling
Sample material and quantity:	tissue hydrolysate: (1) free lung cells, 5oo ul (2) lung homogenate, 2o ul
Results expressed in:	ug
Equipment:	spectrophotometer: model 555, Perkin Elmer GmbH, D-777o Überlingen
Chemicals and reagents:	ammoniumheptamolybdate, no. 1182, HCl, no. 317, E. Merck, D-61oo Darmstadt 1 Tween 2o, no. 822184, Merck-Schuchardt, D-8o11 Hohenbrunn malachit green "G", no. 1B 289 Chroma GmbH, D-7ooo Stuttgart stock solution 1: 42 g/l ammoniumheptamolybdate in 5 mol/l HCl stock solution 2: 2 g/l malachit green stock solution 3: 15 ml/l Tween 2o
Procedure:	according to Itaya, K. and Ui, M., Clinica Chim. Acta 14: 361 (1966)

2501091289

Source: https://www.industrydocuments.ucsf.edu/docs/lghl0000

assay solution:

1 aliquot solution 1 mixed with 3 aliquots solution 3, filtered after standing for 3o minutes. Stable at RT for 3 weeks

photometric determination:

wavelength: 66o nm

standard curve: see FIGURE ...

Scientific version: 22.Sep.81
Text version: 23.Sep.81

250109129o

Source: https://www.industrydocuments.ucsf.edu/docs/lghl0000

Determination of L-lactate concentration

Principle:	photometric determination of NADH formed during the oxidation of L-lactate to pyruvate in the presence of lactate dehydrogenase (EC 1.1.1.27)
Time	
Sampling:	-
Determination:	within ... .. after sampling
Sample material and quantity:	(1) perfusate supernatant, 5o ul (2) muscle tissue extract, deprotreinized, 5o ul
Results expressed in:	(1) mmol/l (2) rate of increase: umol/(min x g muscle WW)
Equipment:	spectrophotometer: model 555, Perkin Elmer GmbH, D-777o Überlingen
Chemicals and reagents:	test set Lactate, enzymatic, no. 256773, standard: Precinorm S, no. 125121, Boehringer Mannheim GmbH, D-68oo Mannheim 31
Procedure:	acc. to Noll, F., in: Bergmeyer, H.U. (ed.): Methoden der enzymatischen Analyse, Vol. 2, Verlag Chemie Weinheim, 3rd ed., 1974, p 1521 photometric determination: wavelength: 334 nm storage until determination: 4 degrees centigrade
Scientific version:	5.Feb.8o
Text version:	8.Oct.81

2501091291

Source: https://www.industrydocuments.ucsf.edu/docs/lghl0000

Determination of L-lactate dehydrogenase (EC 1.1.1.27) activity (macroassay)

Principle:	photometric determination of NADH consumption during reduction of pyruvate to L-lactate
Time	
Sampling:	on day ...
Determination:	on day of sampling
Sample material and quantity:	lung lavage supernatant (3.8x1oE5 m/s2 = 39ooo x g), 5o ul
Results expressed in:	U/l
Equipment:	spectrophotometer: model 555, Perkin Elmer GmbH, D-777o Überlingen
Chemicals and reagents:	monotest LDH optimized method, no. 158186, standard: Precimat E, no. 125o91, Boehringer Mannheim GmbH, D-68oo Mannheim 31
Procedure:	centrifugation of lung lavage: (1) 4.9x1oE3 m/s2 (= 5oo x g), 1o min, o degrees centigrade (2) 3.8x1oE5 m/s2 (= 39ooo x g), 15 min, o degrees centigrade optimized standard method of the Deutsche Gesellschaft für Klinische Chemie, Z. klin. Chem. und klin. Biochem. 8: 658 (197o), ibid. 1o: 182 (1972) photometric determination: wavelength: 34o nm
Scientific version:	7.Oct.8o
Text version:	6.Okt.81

2501091292

Source: https://www.industrydocuments.ucsf.edu/docs/lghl0000

Determination of L-lactate dehydrogenase (EC 1.1.1.27) activity (microassay)

Principle:	photometric determination of NADH consumption during reduction of pyruvate to L-lactate
Time	
Sampling:	on day ...
Determination:	on day of sampling
Sample material and quantity:	(1) free lung cell homogenate (2) lung lavage supernatant (3) serum, 2.5 ul
Results expressed in:	U/l
Equipment:	ABA-1oo Bichromatic Analyzer, Abbott Laboratories, Diagnostics Division, So. Pasadena, California 91o3o, USA
Chemicals and reagents:	monotest LDH optimized method, no. 158186, standard: Precinorm E, no. 125o91, Boehringer Mannheim GmbH, D-68oo Mannheim 31
Procedure:	optimized standard method of the Deutsche Gesellschaft für Klinische Chemie, Z. klin. Chem. und klin. Biochem. 8: 658 (197o), ibid. 1o: 188-189 (1972)

final concentrations of components in assay mixture:

NADH	o.18 mmol/l
phosphate buffer, pH 7.5	5o.oo mmol/l
pyruvate	o.6o mmol/l

each sample determined in duplicates

photometric determination:

wavelength 1: 34o nm
wavelength 2: 38o nm

2501091293

Scientific version: 14.Jul.8o
Text version: 1o.Okt.81

Source: https://www.industrydocuments.ucsf.edu/docs/lghl0000

Isolation of cellular macromolecules

Principle:	phenol extraction and alcohol precipitation of nucleic acids and proteins further purification of DNA with RNAse treatment
Time	
Sampling:	-
Determination:	-
Sample material and quantity:	perfused lungs, 4 lungs per extraction pool
Results expressed in:	-
Equipment:	centrifuge: RC-5B, rotor: SS-34 and GSA, Du Pont Instruments, D-635o Bad Nauheim
Chemicals and reagents:	sodium-triisopropyl naphtaline sulfonate, no. 37o35, sodium-4-aminosalicylate-monohydrate, no. 1322o, Serva Feinbiochemica GmbH und Co. KG, D-69oo Heidelberg 1 butanol-2, no. 963o, phenol, no. 2o6, m-cresol, no. 8o9691, 8-hydroxyquinoline, no. 7o98, sodium acetate, cryst., no. 6267, diethyl ether, no. 923, methanol, no. 6oo9, sodium hydroxide, no. 9137, sodium citrate, no. 6447 and 658o, di-sodiumphosphate dihydrate, no. 658o, E. Merck, D-61oo Darmstadt 1 sodium chloride, no. 6oo9, chloroform, no. 7386, Baker Chemicals, D-6o8o Gross-Gerau

2501091294

Source: https://www.industrydocuments.ucsf.edu/docs/lghl0000

isoamylalcohol, no. 1 1381,
potassium polyvinylsulfate, no. P 6ooo,
Sigma Chemie GmbH,
D-8o21 Taufkirchen

ethanol, denatured with petrol ether,
Werner Hofmann,
D-5ooo Köln 21

ethylgycol, no. 5127,
Carl Roth GmbH,
D-75oo Karlruhe 21

ribonuclease, no. 1o9126,
Boehringer Mannheim GmbH,
D-68oo Mannheim 31

Procedure:

according to Diamond et al., Cancer Res. 27: 89o - 897 (1967) as modified by Kuroki and Heidelberger, Cancer Res. 31: 2168 - 2176 (1971)

extraction medium according to Kirby and Cook, Biochem. J. 1o4: 254-257 (1967), 16 ml/lung

Scientific version: 3.Dec.8o
Text version: 8.Oct.81

2501091295

Source: https://www.industrydocuments.ucsf.edu/docs/lghl0000

Determination of the Michaelis Constant and maximal velocity

Principle:	photometric determination of rate of enzymatic reaction (v) with variable substrate concentrations at constant enzyme, cosubstrate, cofactor, buffer and physical conditions
Time:	-
Sample material and quantity	
LDH (BH, RM):	5 ul test or reference substance solution equivalent to final concentrations of o.o5o, o.o63, o.o83, o.125 and o.25o mmol/l
GLR:	5 ul test or reference substance solution equivalent to final concentrations of o.167, o.2oo, o.25o, o.333, o.5oo and 1.ooo mmol/l
Results expressed in:	1/delta A (a)
Equipment:	ABA-1oo Bichromatic Analyzer, Abbott Laboratories, Diagnostics Division, So, Pasadena, California 91o3o, USA
Chemicals and reagents:	L-lactate dehydrogenase (EC 1.1.1.27) from bovine heart, 55o U/mg protein, 11 mg/ml, no. L 1oo6, L-lactate dehydrogenase (EC 1.1.1.27) from rabbit muscle, LDH 5 isoenzyme, 1ooo U/mg protein, 5ooo U/ml, no. L 2875, glutamic acid, no. G 1251, Sigma Chemie GmbH, D-8o28 Taufkirchen

2501091296

(a) difference of absorption

Source: https://www.industrydocuments.ucsf.edu/docs/lghl0000

glyoxylate reductase (EC 1.1.1.26) from Spinacia oleracea, approx. 55 U/mg protein, 5 mg/ml, no. 1o6151, NAD, grade 2, 98o g/kg, no. 127981, NADH, disodium salt, grade 2, 98o g/kg, no. 128o15, Boehringer Mannheim GmbH, D-68oo Mannheim 31.

potassium dihydrogen phosphate no. 4873, dipotassium hydrogen phosphate, no. 5o99, dipotassium carbonate, no. 4928, E. Merck, D-61oo Darmstadt 1

Procedure:

LDH(BH and RM):

reduction of NAD in the presence of LDH and glyoxylate or piridoxilate at pH 1o.o and 25 degrees centigrade

final concentrations of components in assay mixture:

K2CO3:		o.5o mol/l
glutamate:		62.5o mmol/l
NAD:	(1)	1.16 mmol/l
	(2)	4.65 mmol/l
	(3)	6.98 mmol/l

LDH (BH) or LDH (RM): 2.7x1oE4 U/l

glyoxylate or piridoxilate:	(1)	o.o5o mmol/l
	(2)	o.o63 mmol/l
	(3)	o.o83 mmol/l
	(4)	o.125 mmol/l
	(5)	o.25o mmol/l

determination of change of absorbance during 3rd min after addition of enzyme to substrate, 1st of 3 intervals of 15 s reported

3 to 8 determinations/substrate concentration

GLR:

oxidation of NADH in the presence of GLR and glyoxylate or piridoxilate at pH 6.4 and 25 degrees centigrade

final concentrations of components in assay mixture:

potassium phosphate: 41 mmol/l

2501091297

Source: https://www.industrydocuments.ucsf.edu/docs/lghl0000

NADH: (1) 3.41 mmol/l
(2) 1o.2o mmol/l
(3) 15.3o mmol/l

GLR: 18oo U/l

glyoxylate or
piridoxilate: (1) o.167 mmol/l
(2) o.2oo mmol/l
(3) o.25o mmol/l
(4) o.333 mmol/l
(5) o.5oo mmol/l
(6) 1.ooo mmol/l

triplicate determinations at each substrate concentration

Scientific version: 21.Jan.81
Text version: 8.Oct.81

2501091298

Source: https://www.industrydocuments.ucsf.edu/docs/lghl0000

Preparation of microsomes

Principle:	precipitation of microsomes as a calcium-complex
Time	
Sampling:	on day ...
Determination:	within ... .. after S9 preparation
Sample material and quantity:	S9 fraction, 6 ml
Results expressed in:	-
Equipment:	centrifuge: RC-5B, rotor: SS-34, Du Pont Instruments, D-635o Bad Nauheim homogenizer: Potter-Elvehjem, glass, with motor-driven teflon pestle, Braun Melsungen AG, D-35o8 Melsungen
Chemicals and reagents:	calcium chloride 2-hydrate, no. 2383, potassium chloride, no. 4936, E. Merck, D-61oo Darmstadt 1
Procedure:	according to Litterst, C.L., Mimnaugh, E.G., Reagan, R.L. and Gram, T.E., Life Sciences 17: 813-818 (1975) glassware and equipment in contact with S9 fraction precooled
Scientific version:	23.Mar.8o
Text version:	15.Jul.81

2501091299

Source: https://www.industrydocuments.ucsf.edu/docs/lghl0000

Preparation of microsomes

Principle:	sedimentation of microsomes by centrifugation
Time	
Sampling:	on day ...
Determination:	within ... .. after S9 preparation
Sample material and quantity:	S9 fraction, total amount
Results expressed in:	-
Equipment:	centrifuge: OTD-65, rotor: T-865.1, Du Pont Instruments, D-635o Bad Nauheim homogenizer: Potter-Elvehjem, glass, with motor-driven teflon pestle, Braun Melsungen AG, D-35o8 Melsungen
Chemicals and reagents:	-
Procedure:	9.8x1oE5 m/s2 (= 1ooooo x g) centrifugation for 6o minutes at 4 degrees centigrade glassware and equipment in contact with S9 fraction precooled
Scientific version:	28.Aug.8o
Text version:	13.Jul.81

2501091300

Source: https://www.industrydocuments.ucsf.edu/docs/lghl0000

Determination of muscle contraction

Principle:	determination of the movement of a cotton thread, linked to the pre-loaded muscle, after transduction to an electrical signal
Time:	continuously from start of muscle stimulation
Sample material and quantity:	right HL of individual rats
Results expressed in:	N x m
Equipment:	electrical transducer K 3o, bridge amplifier coupler type 55o A, Watanabe recorder (3 channels) WTR 281, Hugo Sachs Elektronik KG, D-78o1 March-Hugstetten/Freiburg
Chemicals and reagents:	-

Procedure

Registration:	fixation of cotton thread at Tendo M. tricipitis surae (Achillis), connection to preload (8 g) via sheave pulley. Registration of contractions on recorder

Calculation of mechanical parameters

Mean energy per contraction (MEC):

a = mean recorder pen deflection (1oE-2 m)

$$a = \frac{\sum_{n=1}^{4} \frac{A_n + A_{n+1}}{2} \times (t_{n+1} - t_n)}{t_5 - t_1}$$

2501091301

Source: https://www.industrydocuments.ucsf.edu/docs/lghl0000

A_n = recorder pen deflection (1oE-2 m) due to contractions at time t_n

t_n = time (min) after start of perfusion, time intervals: 15 to 3o, 3o to 5o, 5o to 7o and 7o to 9o min

K = power

MEC = K x S = M x g x S

M = mass of preload = 8x1oE-3 kg

g = acceleration due to gravity = 9.8 m/s2

S = distance of muscle contraction and of movement of M (4x1oE-2 m recorder pen deflection equivalent to 1.4x1oE-3 m movement of M)

MEC (N x m) = 8x1oE-3 kg x 9.8 m/s2 x a

$$x \frac{1.4x1oE-3}{4}$$

= a x 2.74x1oE-5 N x m x 2 (a)

Mean power (MP):

MP = MEC x f

f = frequency of contraction = 6o contractions per minute

MP = MEC x 6o

Scientific version: 28.May 8o
Text version: 19.Aug.81

2501091302

(a) both hind limbs stimulated

Source: https://www.industrydocuments.ucsf.edu/docs/lghl0000

Preparation of muscle extract

Principle:	mechanical homogenization and deproteinization of freeze-clamped tissue in perchloric acid, subsequent neutralization and removal of precipitated potassium perchlorate
Time:	5 min after end of perfusion
Sample material and quantity:	freeze-clamped muscle tissue, right HL, approx. 1 g/sample
Results expressed in:	-
Equipment:	micro-dismembrator, teflon capsula, agate bullets, Braun Melsungen AG, D-35o8 Melsungen centrifuge: model J6, rotor: JS-4.2, Beckman Instruments GmbH, D-8ooo München 4o Wollenberger-type freeze-clamp, aluminium, clamping area: 5o cm2, INBIFO, D-5ooo Köln 9o
Chemicals and reagents:	perchloric acid (1 mol/l), no. 519, potassium carbonate (approx. 5 mol), no. 4928, methyl orange, 72o g/l, No. 1322, E. Merck, D-61oo Darmstadt 1
Procedure:	according to Gutmann, J. and Wahlefeld, A.W., in: Bergmeyer, H.U. (ed.): Methoden der enzymatischen Analyse, Vol. 2, Verlag Chemie Weinheim, 3rd edition, 1974, pp. 151o-1514 and

2501091303

Source: https://www.industrydocuments.ucsf.edu/docs/lghl0000

Lamprecht, W., and Trautschold, J., in: Bergmeyer, H.U. (ed): Methoden der enzymatischen Analyse, Vol. 2, Verlag Chemie Weinheim, 3rd ed., 1974, p. 2155

Homogenization: tissue sample frozen in liquid nitrogen, filled into capsula (minus 196 degrees centigrade) and shaken 3 times for 1o s with maximal amplitude

Scientific version: 24.Apr.8o
Text version: 8.Oct.81

2501091304

Source: https://www.industrydocuments.ucsf.edu/docs/lghl0000

Muscle stimulation

Principle:	direct electric stimulation
Time:	start of stimulation immediately before end of preperfusion, continuous stimulation cycles until end of perfusion
Sample material and quantity:	-
Results expressed in:	-

Equipment

Generator/modulators:	stimulator with frame unit HG 1ooG, clock unit HG 1o1B, control unit HG 1o2B, triple delay HG 1o3B, pulse unit HG 1o8B and isolators HG 2o3, HI-MED Instruments Limited, Reading/Berkshire, United Kingdom
Electrodes:	E-Z-HOOK, test connectors, no. X11, Gonda Elektronik, D-7o12 Fellbach

Procedure

Fixation of electrodes:	electrode "clamped" onto Nerv. ischiadicus at a distance of 5 mm to the M. gastrocnemius, 2nd electrode "clamped" onto the Tendo M. tricipitis surae (Achillis) close to the M. gastrocnemius
Mode:	direct stimulation
Frequency:	1 impuls/s
Duration of individual stimulation:	1.5 ms
Voltage:	3 V

2501091305

Scientific version: 27.Nov.8o
Text version: 8.Oct.81

Source: https://www.industrydocuments.ucsf.edu/docs/lghl0000

Determination of O-demethylase (EC 1.14.99.15) activity

Principle:	photometric determination of p-nitrophenol formed by demethylation of p-nitroanisol during incubation with 8.8x1oE4 m/s2 (= 9ooo x g) supernatant of tissue homogenates or microsomes
Time	
Sampling:	on day ...
Determination:	within ... .. after preparation of 8.8x1oE4 m/s2 (= 9ooo x g) supernatant or microsomes
Sample material and quantity:	liver: 4o ul of 8.8x1oE4 m/s2 (= 9ooo x g) supernatant equiv. to approx. 3 mg protein, linear range: o to 9 mg protein kidney: o.3 to o.6 mg microsomal protein, linear up to 1 mg protein lung: 2 to 4 mg microsomal protein, linear up to 7 mg protein
Results expressed in:	pmol p-nitrophenol/(mg protein x min)
Equipment:	centrifuge: model J6, rotor: JS-4.2, Beckman Instruments GmbH, D-8ooo München 4o high pressure liquid chromatograph: model 1o84 B, Hewlett Packard, D-75oo Karlsruhe column: no. RP-18, 25o mm x 4 mm, Knauer, D-637o Oberursel

2501091306

Source: https://www.industrydocuments.ucsf.edu/docs/lghl0000

Chemicals and reagents:

glucose-6-phosphate dehydrogenase (G6PDH, EC 1.1.1.49), no. 197726,
glucose-6-phosphate (G6P), no. 127o27,
NADP, no. 128o31,
NAD, no. 127981,
Boehringer Mannheim GmbH,
D-68oo Mannheim 31

trichloroacetic acid (TCA), no. 8o7,
magnesium chloride, no. 5833,
E. Merck,
D-61oo Darmstadt 1

bovine serum albumin (BSA), no. A 4378,
Trizma base (Tris), no. T 15o3,
Sigma Chemie GmbH,
D-8o21 Taufkirchen

acetonitrile, no. 8143,
Baker Chemicals,
D-6o8o Gross-Gerau

p-nitrophenole (p-Np), no. 82o896,
p-nitroanisole (p-Na), no. 8o6228,
Merck-Schuchardt,
D-8o11 Hohenbrunn

Procedure:

according to Netter, K.J., Naunyn-Schmiedeberg's Arch. Exp. Path. Pharmakol. 238: 292 (196o) modified by Inbifo for HPLC

final concentrations of components in assay mixture:

protein	... g/l
G6P	2.o8 mmol/l
G6PDH	o.4 U/ml
BSA	o.67 g/l
NADP	o.31 mmol/l
NAD	o.36 mmol/l
magnesium chloride	4.17 mmol/l
p-nitroanisole	o.83 mmol/l

standard curve: see FIGURE ...

Scientific version: 15.Jul.8o
Text version: 9.Oct.81

2501091307

Source: https://www.industrydocuments.ucsf.edu/docs/lghl0000

Determination of L-ornithine decarboxylase (EC 4.1.1.17) activity

Principle:	radiometric determination of 14C-carbon dioxide formed by decarboxylation during incubation of DL-14C-ornithine with centrifuged epidermis homogenate (postmicrosomal supernatant)
Time	
Sampling:	on day ...
Determination:	within ... .. after preparation of microsomes
Sample material and quantity:	(1) 9.8x1oE5 m/s2 (= 1ooooo x g) postmicrosomal supernatant of epidermis homogenate pool, .. mice per pool, approx. o.3 mg protein per assay linear range: o.1 to 2.o mg protein (2) 9.8x1oE5 m/s2 (= 1ooooo x g) postmicrosomal supernatant of tracheal mucosa homogenates pool, .. rats per pool, approx. .. mg protein per assay
Results expressed in:	(1) specific activity: U/mg protein (2) skin activity: U/g skin
Equipment:	incubation vessels: 25 ml Erlenmeyer-flasks with center wells, Faust GmbH, D-5ooo Köln 9o screw caps with fluorene elastomer sealing: Gummiwerke Verneret, D-5o24 Pulheim 2 rotary shaking water bath: WTR-1, Infors AG, CH-4o51 Basel, Switzerland thermometer: R4o/2, digital, Messgerätewerk Lauda, Dr. R. Wobser KG, D-697o Lauda-Königshofen

2501091308

Source: https://www.industrydocuments.ucsf.edu/docs/lghl0000

scintillation counter: Mark 3 model 68oo,
W. Zinsser,
D-6ooo Frankfurt/Main 5o

Chemicals and reagents:

DL-14C-ornithine 1-hydrochloride, no. NEC-469,
carbon dioxide absorber:
ethanolamine/ethyleneglycol, 2/1 (1/1),
ethanolamine, no. NEF-954,
New England Nuclear Chemicals GmbH,
D-6o72 Dreieich

sodium dihydrogen phosphate 1-hydrate, no. 6346,
disodium hydrogen phosphate 2-hydrate, no. 658o,
citric acid, no. 244,
E. Merck,
D-61oo Darmstadt 1

1,4-dithioerythrit, no. 223662,
Boehringer Mannheim GmbH,
D-68oo Mannheim 31

pyridoxal-5-phosphate, no. P 9255,
L-ornithine hydrochloride, no. O 2375,
Sigma Chemie GmbH,
D-8o21 Taufkirchen

ethyleneglycol monomethyl ether, no. 18546-9,
Ega Chemie,
D-7924 Steinheim/Albuch

Procedure:

according to O`Brien, T.G., Simsiman, R.C. and Boutwell, R.K., Cancer Res. 35: 1662-167o (1975) with modifications by INBIFO

final concentrations of components in assay mixture (a):

protein (b) approx.	o.15 g/l
pyridoxal-5-phosphate	o.21 mmol/l
1,4-dithioerythrit	o.6o mmol/l
sodium phosphate	25.oo mmol/l
EDTA	o.o1 mmol/l
L-ornithine	o.1o mmol/l
DL-14C-ornithine	1.85x1oE7 Bq/l (= o.5 mCi/l)

2501091309

(a) contribution by homogenization buffer: compounds included
(b) postmicrosomal supernatant

Source: https://www.industrydocuments.ucsf.edu/docs/lghl0000

determination of radioactivity:
see ...

reaction immediately stopped after incubation (a) with 1 ml citric acid (final concentration: o.67 mol/l)

Calculation:

$$\frac{\text{radioactivity (dpm)} \times (\text{labeled (ug)} + \text{unlabeled L-ornithine})}{\text{constant (dpm/pCi)} \times \text{L-14C-ornithine (ug)} \times \text{time (h)} \times \text{protein (mg/sample)} \times \text{spec. radioact. (b) (pCi/pmol)}}$$

1 u = 1 nmol CO2/h

standard curve: see FIGURE ...

Scientific version: 1.Feb.81
Text version: 24.Sep.81

(a) blank stopped before start of incubation
(b) DL-14C-ornithine

2501091310

Source: https://www.industrydocuments.ucsf.edu/docs/lghl0000

Organ perfusion

Principle:	in situ recirculating (constant volume) perfusion of organ with oxygenated cell-free synthetic medium
Time:	-
Sample material and quantity:	lungs from anesthetized rats, perfused individually
Equipment:	perfusion apparatus: all glass system comprising oxygenator, reservoir and tubes peristaltic pumps, E 25, Reichelt Chemie Technik, D-69oo Heidelberg 1 LP-1A, Amicon GmbH, D-581o Witten
Chemicals and reagents:	perfusate: half-synthetic medium according to Shimassek, Life Sci. 11: 629 - 634 (1962), based on Krebs-Ringer-bicarbonate buffer, pH 7.6 oxygenation: 95o ml/l oxygen, 5 ml/l carbon dioxide bovine serum albumin, no. A 45o3, Sigma Chemie GmbH, D-8o21 Taufkirchen 3H-benzo(a)pyrene, no. NET-572, New England Nuclear, D-6o72 Dreieich

Procedure

Anesthesia:	i.p. injection of urethane, 1.2 g/kg body weight as a 2o o/o solution in saline

2501091311

Source: https://www.industrydocuments.ucsf.edu/docs/lghl0000

Heparinization:	i.v. injection of heparin, 1o mg/kg body weight as o.4 percent solution in saline
	volume of perfusate: 3o ml
	pump rate:(1) 2o ml/min (2) 1o ml/min = constant volume perfusion
	oxygenation: 37 degrees centigrade, 6 to 7 l gas/h
	organ entry: via pulmonary artery
	organ exit: via opened heart
Pre-perfusion:	15 min-perfusion in the absence of benzo(a)pyrene
Tritium incorporation:	45 min, 1 micromolecule benzo(a)pyrene/l perfusate equivalent to 1mCi per 3o ml
Post-perfusion:	approx. 3 min with oxygenated Krebs-Ringer buffer
Storage of organ:	at minus 8o degrees centigrade

Scientific version: 3.Dec.79
Text version: 8.Oct.81

2501091312

Source: https://www.industrydocuments.ucsf.edu/docs/lghl0000

Organ perfusion

Principle:	in situ recirculating, volume-constant perfusion of the HL with oxygenated, ery. containing, synthetic medium
Time:	on day ...
Sample material and quantity:	both HL
Results expressed in:	-
Equipment:	perfusion apparatus: see FIGURE .. peristaltic pumps: E 25, Reichelt Chemie Technik, D-69oo Heidelberg 1 LP-1A, Amicon GmbH, D-581o Witten thermostat: RM3, Messgeräte-Werk Lauda, Dr. R. Wobser KG, D-697o Lauda-Königshofen
Chemicals and reagents:	perfusion medium according to Ruderman et al., Biochem. J. 124: 639-651 (1971). Perfusion medium with pyruvate, based on Krebs-Ringer bicarbonate buffer, composition see TABLE .., pH adjusted with 1oo g/l sodium hydrogen carbonate solution to 7.35 ± o.o2 after gas and temperature equilibration gases: oxygen, pressurized, nitrogen, pressurized, pure (2 ring-marks) carbon dioxide, pressurized Elbert, D-5ooo Köln 1

2501091313

Source: https://www.industrydocuments.ucsf.edu/docs/lghl0000

bovine serum albumin, no. o6o4,
Baker Chemicals,
D-6o8o Gross-Gerau

glass wool, acetone washed, no. 4o86,
D(+)-glucose 1-hydrate, no. 8346,
magnesium sulfate 7-hydrate,
no. 5886,
potassium chloride, no. 4936,
calcium chloride 2-hydrate,
no. 2382,
sodium chloride, no. 64o4,
potassium dihydrogen phosphate,
no. 4873,
sodium hydrogen carbonate, no. 6329,
E. Merck,
D-61oo Darmstadt 1

Procedure

Temperature (a):	39.o ± o.o1 degrees centigrade
Volume of perfusate:	13o ml minus 3o ml discarded during preperfusion
Pump rate:	pump 1 (see FIGURE .): 2o ml/min pump 2 (see FIGURE .): 1o ml/min (b)
Gas equilibration:	6 to 7 l gas/h, prewarmed to 39 degrees centigrade, ery. containing perfusion medium equilibrated with starting gas during 2o to 3o min preceding the perfusion
Oxigenator funnel rotation:	approx. 1o rpm
Organ entry:	via A. abdominalis
Organ exit:	via V. cava inferior
Preperfusion:	approx. 3 min, perfusate not recirculated and 1st 3o ml discarded

Scientific version: 3o.Jan.8o
Text version: 24.Sep.81

2501091314

(a) in thermostat water reservoir
(b) volume-constant perfusion

Source: https://www.industrydocuments.ucsf.edu/docs/lghl0000

Determination of osmolality

Principle:	determination of freezing temperature depression
Time	
Sampling:	on day ..
Determination:	within .. hours after sampling
Sample material and quantity:	(1) lavage media, 15o ul (2) cell suspensions, 15o ul (3) lavage supernatant, 15o ul (4) perfusate supernatant, 15o ul
Results expressed in:	mmol/kg
Equipment:	osmometer: digital, KG Wissenschaftlicher Gerätebau Dr. Knauer und Co. GmbH, D-1ooo Berlin 37 pipettes: type 471o, Netheler und Hinz, D-2ooo Hamburg 63
Chemicals and reagents:	calibration solution: 4oo mmol/kg, KG Wissenschaftlicher Gerätebau Dr. Knauer und Co. GmbH, D-1ooo Berlin 37
Procedure:	aqueous sample cooled below freezing point and freezing initiated by vibration. Determination of resulting freezing temperature by means of electronic thermistor. Automatic calculation of osmolality from freezing temperature depression (minus 1.858 degrees centigrade equivalent to 1 mol/kg).
Scientific version:	16.Sep.8o
Text version:	24.Sep.81

2501091315

Source: https://www.industrydocuments.ucsf.edu/docs/lghl0000

Determination of oxygen supply and consumption

Principle: polarographic determination of oxygen partial pressure in perfusate before and after perfused HL, oxygen concentration calculated on the basis of the Henry-Dalton law

$$(1) = \frac{(O2,\ HB\text{-bound} + O2,\ phys.\ diss.)_B}{time \times muscle\ mass}$$

$$(2) = \frac{(O2,\ HB\text{-bound} + O2,\ phys.\ diss.)_A}{time \times muscle\ mass}$$

B: before perfused HL
A: after perfused HL
oxygen supply = (1)
oxygen consumption = (1) - (2)

$$\text{oxgen extraction} = \frac{\text{consumption} \times 1oo}{\text{supply}}$$

Time: -

Sample material and quantity: perfusate, o.5 ml before and after perfused HL, sites of sampling: see FIGURE ..

Results expressed in:

(1) supply, consumption or extraction at the time of sampling
(2) mean supply, mean consumption or mean extraction of each interval (= mean of start and end of interval)

supply and consumption: umol/(min x g muscle WW)

extraction: o/o

Equipment: Astrup Micro Equipment: AME1 or BMS3 MK2 and Digital Acid-base Analyzer PHM7 MK2
Radiometer A/S,
DK-24oo Copenhagen, Denmark
via
Hillerkus,
D-415o Krefeld-Bochum

2501091316

Source: https://www.industrydocuments.ucsf.edu/docs/lghl0000

tuberculin syringes, polypropylene,
1 ml, no. 941oooo,
Asik Hospital Supplies,
Denmark

Procedure

according to Clark, L.C., Tr. Am. Soc. for Art. Int. Organs 2: 41 (1956)

Calculations:

(1) O2, phys. dissolved ((O2)P):

$$(O2)P = pO2 \times \frac{o.o24 \times 1ooo}{76o}$$

pO2: oxygen partial pressure (mm Hg)

(2) O2, HB-bound ((O2)HB):

$$(O2)HB = \frac{sO2corr. \times 1.39 \times (HB)}{1oo}$$

sO2corr.: oxygen saturation corrected for temperature and pH according to Severinghaus, W. in: Altman, P.L., Dittmer, D.S. (Eds.): Blood and other body fluids, Washington DC: Fed. Amer. Soc. Expl. Biol. 1961 (see FIGURES . to .)

sO2corr. pO2/(pH corr. factor x temperature corr. factor)

Hb: hemoglobin concentration (g/l)

(3) oxygen supply (OS):

$$OS = \frac{((O2)Hb + (O2)p) \times FR}{22.4 \times BW/1o}$$

BW = body weight (g)

FR = flow rate (ml/min)

(4) oxygen consumption (OC):

$$OC = (OS)_B - (OS)_A$$

2501091317

Scientific version: 5.Feb.8o
Text version: 15.Jun.81

Source: https://www.industrydocuments.ucsf.edu/docs/lghl0000

Preparation of perfusate supernatant

Principle:	separation of perfusate supernatant from erythrocytes by centrifugation
Time	
Sampling:	-
Determination:	within 2 min after sampling
Sample material and quantity:	perfusate, 1.o ml
Results expressed in:	-
Equipment:	centrifuge: type "Eppendorf", no. 5412, micro vials: type "Eppendorf", no. 381o, polypropylene, Netheler und Hinz, D-2ooo Hamburg 63
Chemicals and reagents:	-
Procedure	
Centrifugation:	9.76x1oE4 m/s2 (= 995o x g) for 2 min at RT, transfer of perfusate supernatant to fresh micro vial
Storage:	at o to 4 degrees centigrade
Scientific version:	5.Feb.8o
Text version:	24.Sep.81

2501091318

Source: https://www.industrydocuments.ucsf.edu/docs/lghl0000

Determination of phagocytic index

Principle:	impedance change of counting (Coulter principle) of fungal spores phagocytized (attached or engulfed)
Time	
Sampling:	on day ...
Determination:	within 4 h after start of harvest of free lung cells
Sample material and quantity:	free lung cells, o.5x1oE6 macrophages per incubation dish
Results expressed in:	spores/macrophages
Equipment:	incubation dishes: petri dishes, no. 5221, 35 mm x 1o mm, Lux Scientific Corporation, California, USA
	incubator: W.C. Heraeus GmbH, D-325o Hanau
	sonifier: B-15, titanium microtip, Branson Sonic Power Company, Danbury, Connecticut, USA
	particle counter: Coulter Counter model ZF, Coulter Electronics Limited, D-415o Krefeld
	centrifuge: model J6, rotor: JS-4.2, Beckmann Instruments GmbH, D-8ooo München 4o
	microscope: 4oo-fold magnification, standard 14, objective: PL 4o/o.65, Carl Zeiss, D-7o82 Oberkochen

2501091319

Source: https://www.industrydocuments.ucsf.edu/docs/lghl0000

Chemicals and reagents:

cell suspension medium:
medium 199 (modified), with Earle's salts, without sodium bicarbonate, without glutamine, with 2o mmol/l Hepes buffer and addition of Penicillin and Streptomycin,
no. 12-2o4-49,
fetal bovine serum, filtersterilized, mycoplasma and virus screened,
no. 29-1o1-49 (4-55D),
Flow Laboratories GmbH,
D-53o9 Meckenheim

Isoton 2,
Zaponin,
Coulter Electronics Limited,
Hertfordshire, United Kingdom

spores of the basidiomycete Geastrum triplex Jungh, diameter: approx. 5 um,
University of Gothenburg, Sweden

phosphate buffered saline (PBS), Instamed PBS, calcium-and magnesium-free, no. 182-1, pH 7.2,
Seromed GmbH,
D-8ooo München 71

Triton X-1oo, no. 37238,
penicillin, potassium salt, no. 31749,
streptomycin sulfate, no. 355oo,
Serva Feinbiochemica GmbH and Co.,
D-69oo Heidelberg 1

Procedure

Preparation of fungal spores:

spores suspended and boiled in o.o3 g/l Zaponin for 5 min, suspension centrifuged at 3.2x1oE4 m/s2 (= 33oo x g) for 1o min. After 5 subsequent washes of the spores in bidistilled water, spores resuspended to a concentration of 1.4 to 1.7x1o E7/ml bidistilled water and stored at minus 3o degrees centigrade. Prior to addition to free lung cells, thawed spores centrifuged at 3.2x1oE4 m/s2 (= 33oo x g) and resuspended in cell suspension medium with 1oo ml/l fetal bovine serum to a concentration of 1.4 to 1.7x1oE7/ml

2501091320

Source: https://www.industrydocuments.ucsf.edu/docs/lghl0000

Phagocytosis assay:

a defined number of free lung cells in medium 199 mixed and incubated in a petri dish for 9o min at 37 degrees centigrade (control: 4 degrees centigrade) with 1.5x1oE7 pretreated fungal spores in the presence of 5o ml/l fetal bovine serum

during incubation and phagocytosis of spores most phagocytic cells adhered to the bottom of the dish and were retained there during the subsequent 2 washes of the cells with PBS. Retained cells destroyed by sonication in the presence of the detergent Triton X-1oo, spores (adherent to or engulfed by these cells) released into supernatant and counted with particle counter.

Scientific version: 19.Sep.8o
Text version: 3o.Sep.81

2501091321

Source: https://www.industrydocuments.ucsf.edu/docs/lghl0000

Determination of phagoluminescence

Principle:	determination of luminescence during the incubation of suspended free lung cells with Zymosan particles in the presence of luminol
Time	
Sampling:	on day ...
Determination:	within .. hours after start of harvest of free lung cells
Sample material and quantity:	free lung cells, 5x1oE5 macrophages/ determination
Results expressed in:	(1) log cpm/1oE3 macrophages (2) cpm/1oE3 macrophages
Equipment:	pH-meter: PW 94o9, glass electrode: CA/14o2 Philips GmbH, D-35oo Kassel
	osmometer: no. 77o58, KG Wissenschaftlicher Gerätebau Dr. Knauer und Co. GmbH, D-1ooo Berlin 37
	centrifuges: model J6, rotor: JS-4.2, Beckmann Instruments GmbH, D-8ooo München 4o
	model 5412, Netheler und Hinz, D-2ooo Hamburg 63
	scintillation counter: Mark 3 model 688o,
	scintillation vials: polypropylene, no. 714, W. Zinsser, D-6ooo Frankfurt/Main

2501091322

Source: https://www.industrydocuments.ucsf.edu/docs/lghl0000

microscope: standard 14,
objective: PL 4o/o.65,
Carl Zeiss,
D-7o82 Oberkochen

Coulter mixer C5,
Coulter Electronics Limited,
D-415o Krefeld

Chemicals and reagents:

cell suspension medium:
Eagle's essential medium, according to Eagle, H., Science 13o: 342 (1959), 1o-fold concentrate, no. 14-33o-54,

fetal bovine serum, filter-sterilized, mycoplasm and virus screened, no. 29-1o1-49 (4-55D),
Flow Laboratories GmbH,
D-53o9 Meckenheim

glutamine, no. 22942,
Hepes, no. 25245,
penicillin, no. 31749
streptomycin, no. 355oo,
Serva Feinbiochemica GmbH und Co.,
D-69oo Heidelberg 1

sodium bicarbonate, no. 6329,
E. Merck,
D-61oo Darmstadt 1

Luminol (5-amino-2,3-dihydro-1,4-phthalazinedione), no. A 8511,

Zymosan A from Saccharomyces cerevisiae, no. Z 425o,
Sigma Chemie GmbH,
D-8021 Taufkirchen

Procedure

Determination:

free lung cells, luminol and Zymosan particles suspended or dissolved in cell suspension medium at RT. Background luminescence of a known number of free lung cellsin cell suspension medium (1 ml/5x1oE5 macrophages) with luminol determined with the photomultiplier of a scintillation counter system 1o minutes prior to the addition of Zymosan particles. Luminescence of the incubation mixture determined at approx. 1o min intervals during the subsequent 18o min.

2501091323

Source: https://www.industrydocuments.ucsf.edu/docs/lghl0000

Calculation:	substraction of background luminescence from luminescence maximum. Calculation of luminescence for 1x1oE6 macrophages.
Scientific version:	23.Sep.8o
Text version:	24.Sep.81

2501091324

Source: https://www.industrydocuments.ucsf.edu/docs/lghl0000

Determination of potassium concentration

Principle:	flame photometric determination of the intensity of the characteristic potassium emission in relation to the emission of an internal lithium standard
Time	
Sampling:	-
Determination:	within ... .. after sampling
Sample material and quantity:	(1) serum, 24 ul (2) urine, 24 ul (3) perfusate supernatant, 24 ul
Results expressed in:	mmol/l
Equipment:	KLiNa-flame photometer, no. 65271o, Beckman Instruments GmbH, D-8ooo München 4o
Chemicals and reagents:	internal lithium-standard: 15 mmol/l, no. 663229 sodium/potassium standard: sodium: 14o mmol/l, potassium: 5 mmol/l, no. 663223, Beckman Instruments GmbH, D-8ooo München 4o
Procedure:	according to manufacturer's recommendations storage until determination: 4 degrees centigrade
Scientific version:	6.Mar.79
Text version:	19.Aug.81

2501091325

Source: https://www.industrydocuments.ucsf.edu/docs/lghl0000

Preparation of protein for SDS polyacrylamide electrophoresis

Principle:	dissociation of protein subunits with a sulfhydryl compound (beta -mercaptoethanol) and denaturation as well as surface coating with a negatively charged detergent (SDS)
Time	
Sampling:	on day ..
Determination:	within ... days after sampling
Sample material and quantity:	tissue homogenate or 2.9x1oE5 m/s2 (= 3oooo x g) pellet, 1 mg protein
Results expressed in:	-
Equipment:	micro vials: no. 381o, thermostat: no. 34o1, Netheler und Hinz GmbH, D-2ooo Hamburg 63 whirlmix: no. 34526, Cenco Instrumenten, Breda, The Netherlands analytical balance: model 2oo1 MP, Sartorius GmbH, D-34oo Göttingen pH meter: no. PW 94o9, glass electrode: no. CA/14o2, Philips GmbH, D-35oo Kassel
Chemicals and reagents:	disodium hydrogenphosphate 2-hydrate, no. 658o, sodium dihydrogenphosphate 1-hydrate, no. 6346, beta-mercaptoethanol, no. 8o574o, glycerine, 87o ml/l, no. 4o94, bromophenol blue, no. 8122, E. Merck, D-61oo Darmstadt 1 sodium dodecyl sulfate, no. 2o76o, Serva Feinbiochemica GmbH, D-69oo Heidelberg 1

2501091326

Source: https://www.industrydocuments.ucsf.edu/docs/lghl0000

iodoacetamide, no. I 6125,
Sigma Chemie GmbH,
D-8o21 Taufkirchen

Procedure:

protein incubated for 5 min at 1oo degrees centigrade in SDS phosphate buffer, pH 7.o, containing beta-mercaptoethanol. Thereafter addition of bromophenol blue as tracking dye and glycerine to increase sample density. Further incubation with iodoacetamide at 37 degrees centigrade for 15 min to prevent aggregation of subunits.

final concentrations of components in incubation mixture:

protein	1 g/l
SDS	6.5 g/l
phosphate buffer, pH 7.o	47 mmol/l
beta-mercaptoethanol	125 ml/l
bromophenol blue	o.15 g/l
glycerine	25o ml/l
iodoacetamide	62.5 mmol/l

storage:

minus 2o degrees centigrade, stability unlimited

Scientific version: 7.Jan.81
Text version: 9.Oct.81

25010913 27

Source: https://www.industrydocuments.ucsf.edu/docs/lghl0000

Determination of protein concentration (Bio-Rad method)

Principle:	photometric determination of a complex formed between proteins and Coomassie Brillant Blue G-25o. The absorbance maximum for an acidic solution of the dye shifts from 465 nm to 495 nm when binding to protein occurs.
Time	
Sampling:	on day ..
Determination:	within ... .. after sampling
Sample material and quantity:	(1) purified nucleic acid fractions (2) free lung cell lysate, 1oo ul
Results expressed in:	g/l
Equipment:	spectrophotometer: model 555, Perkin Elmer GmbH, D-777o Überlingen
Chemicals and reagents:	Bio-Rad Protein Kit, no. 5oo-ooo6, standard protein (bovine gamma globulin), no. 5oo-ooo5, Bio-Rad Laboratories, D-8ooo München 5o
Procedure:	according to Bradford, M. M., Anal. Biochem. 72: 248 (1976), photometric determination: wavelength: 595 nm standard curve: see FIGURE ..
Scientific version:	17.Mar.8o
Text version:	24.Sep.81

2501091328

Source: https://www.industrydocuments.ucsf.edu/docs/lghl0000

Determination of protein concentration (Biuret method)

Principle:	photometric determination of a dye complex formed between peptide bonds and the Biuret reagent
Time	
Sampling:	on day ...
Determination:	within ... .. after sampling
Sample material and quantity:	(1) serum, 1o ul (2) S9 fraction, 1o ul (3) postmicrosomal supernatant, 1o ul (4) phenol-extracted protein, 1o ul (5) microsomal pellet, 1o ul
Results expressed in:	g/l
Equipment:	ABA-1oo Bichromatic Analyzer, Abbott Laboratories, Diagnostics Division, So. Pasadena, California 91o3o, USA
Chemicals and reagents:	test set Total protein (Biuret method), no. 124281, standard protein: Precimat, no. 1256o1, Boehringer Mannheim GmbH, D-68oo Mannheim 31
Procedure:	according to Weichselbaum, T. E., Amer. J. Clin. Path. 16: 4o (1946), adapted by INBIFO to the bichromatic analyzer.

final concentrations of components in assay mixture:

sodium hydroxide	o.1 mol/l
potassium sodium tartrate	15.7 mmol/l
potassium jodide	14.7 mmol/l
copper sulfate	5.9 mmol/l

2501091329

Source: https://www.industrydocuments.ucsf.edu/docs/lghl0000

photometric determination:

wavelength 1: 55o nm
wavelength 2: 65o nm

each sample determined in duplicates

standard curve: see FIGURE ...

Scientific version: 28.Mar.79
Text version: 9.Sep.81

2501091330

Source: https://www.industrydocuments.ucsf.edu/docs/lghl0000

Determination of protein concentration (Lowry-method)

Principle:	complex formation of protein with copper ions in alkaline solution and reduction of the phosphomolybdic-phosphotungstic reagent by the copper treated protein
Time	
Sampling:	on day ...
Determination:	within ... .. after sampling
Sample material and quantity:	(1) epidermal sediment, 5o to 1oo ul (2) epidermal microsomal pellet, 3o to 5o ul (3) epidermal supernatant, 1o to 5o ul
Results expressed in:	g/l
Equipment:	spectrophotometer: model 555, Perkin Elmer GmbH, D-777o Überlingen test tubes: no. 611-53, Sovirel, Le Vallois Perret, France pipettes: type "Eppendorf Varipette", no. 471o, Netheler und Hinz, D-2ooo Hamburg 63
Chemicals and reagents	
Chemicals:	coppersulfate, no. 2791, sodium-potassium tartrate 4-hydrate, no. 8o85, sodium hydroxide, no. 9137, sodium carbonate 1o-hydrate, no. 63, Folin-Ciocalteu-Phenol reagent, no. 9oo1, E. Merck, D-61oo Darmstadt 1 standard protein: Precimat, no., 1256o1, Boehringer Mannheim GmbH, D-68oo Mannheim 31

2501091331

Source: https://www.industrydocuments.ucsf.edu/docs/lghl0000

Reagents:

(1) 2o g/l sodium carbonate 1o-hydrate in o.1 mol/l NaOH
(2) 27 g/l sodium-potassium tartrate 4-hydrate in bidistilled water
(3) 1o g/l copper sulfate in bidistilled water
(4) 1o g/l SDS in o.1 mol/l NaOH
(5) alkaline copper reagent (store not more than 12 hours):
1oo ml solution (1)
1 ml solution (2)
1 ml solution (3)
(6) diluted Folin-Ciocalteu-Phenol reagent:
1 plus 1 with bidistilled water (prepared just prior to use)
(7) 5o to 1oo ul epidermal sediment in 145o and 14oo ul solution (4), respectively,
incubation by 75 degrees centigrade for 15 min
(8) protein standard:
1 mg standard protein per ml solution (4)

Procedure:

in the order given the following volumes of solutions are pipetted into glass test tubes:

(1) standard protein solution (1o, 2o, 4o, and 8o ul)
or
5o to 1oo ul of SDS-diluted epidermal protein (7)
or
3o to 1oo ul epidermal microsomal pellet
or
1o to 1oo epidermal supernatant, filled to 1oo ul with solution (4)
(2) 15o ul bidistilled water
(3) 25o ul 1 mol/l NaOH
(4) 2.5 ml alkaline copper reagent (5)

After 1o minutes at Rt, o.25 ml diluted Folin-Ciocalteu-Phenol reagent added and mixed at once.

Mixtures left to stand for at least 3o minutes at RT.

photometric determination:

wavelength: 72o nm

standard curve: see Figure ...

2501091332

Source: https://www.industrydocuments.ucsf.edu/docs/lghl0000

References: Lowry, O.H., Rosebrough, N.J., Farr, A.L., Randall, R.J., J. Biol. Chem. 193 : 165 (1951)

Scientific version: 28.Sep.81
Text version: 12.Oct.81

2501091333

Source: https://www.industrydocuments.ucsf.edu/docs/lghl0000

Determination of pyruvate concentration

Principle:	photometric determination of NADH oxidation during formation of L-Lactate from pyruvate in the presence of lactate dehydrogenase (EC 1.1.1.27)
Time	
Sampling:	-
Determination:	within ... . after sampling
Sample material and quantity:	(1) perfusate, deproteinized, 1 ml (2) tissue extract, deproteinized, 1 ml
Results expressed in:	(1) umol/l or mmol/l (2) nmol/l
Equipment:	spectrophotometer: model 555, Perkin Elmer GmbH, D-777o Überlingen
Chemicals and reagents:	test set Pyruvate, no. 124982, Boehringer Mannheim GmbH, D-68oo Mannheim 31
Procedure:	acc. to Czok, R. and Lamprecht, N., in: Bergmeyer, H.U. (ed.): Methoden der enzymatischen Analyse, Vol. 2, Verlag Chemie Weinheim, 3rd ed., 1974, pp. 1491 photometric determination: wavelength: 34o nm
Scientific version:	1o.Apr.8o
Text version:	28.Sep.81

2501091334

Source: https://www.industrydocuments.ucsf.edu/docs/lghl0000

Determination of radioactivity (liquid scintillation method)

Principle:	determination of the light emission in mixtures of the radioactive sample with scintillation cocktail
Time:	within ... .. after .....
Sample material and quantity:	(1) lung homogenate, 1o ul (2) fractionated cellular macromolecules, 1o ul (3) urine, 1 ml (4) carbon dioxide absorber, 2oo ul
Results expressed in:	dpm (blank already substracted)
Equipment:	liquid scintillation counter: Mark 3 model 68oo, W. Zinsser, D-6ooo Franfurt/Main 5o counting vials: polyethylene, with screw caps, no. 3o714o1, W. Zinsser, D-6ooo Frankfurt/Main 5o
Chemicals and reagents:	scintillation cocktail: Insta gel, no. 6o13oo9 or permafluor 5, no. 6o13129, Packard Instrument Company, D-6ooo Frankfurt/Main
Procedure:	sample mixed with 1o ml scintillation cocktail and mixture equilibrated to counting temperature (approx. 9 degrees centigrade) in the dark automatic quench correction with experimentally determined correction standards counting time: variable program: 1 (3H) or 2 (14C) cumulative counting (4oooo cpm, 2 x sigma: 1 o/o)
Scientific version:	1.Feb.81
Text version:	28.Jan.81

2501091335

Source: https://www.industrydocuments.ucsf.edu/docs/lghl0000

Radioimmunoassay determination of cotinine

Principle:	binding of antigen (cotinine) to specific antibody (anti-cotinine), one of them being attached to a solid substratum, the other one being labeled. Competition of the test sample antigen for the antibody binding sites quantification of unknown cotinine concentrations by comparison with a standard curve obtained with known concentrations of cotinine
Time:	-
Sample material and quantity:	plasma or test compound solution
Results expressed in:	ng/ml
Equipment:	liquid scintillation counter: Mark 3 model 6800, W. Zinsser, D-6000 Frankfurt/Main PVC microtiter plates, immulon, M 124 A, Cooke via Dynatech, D-7310 Plochingen
Chemicals and reagents:	125I-N-(p-hydrox phenetyl)-trans-cotinine carboxamide, Institut für Biopharmazeutische Mikroanalytik, Dr. G. Neurath, D-2000 Hamburg 56 human serum albumin, essentially globulin free, no. A 8763, Sigma Chemie GmbH, D-8021 Taufkirchen 125I, labeled by New England Nuclear, D-6072 Dreieich anti-cotinine antiserum (rabbit), monoclonal anti-cotinine antibodies, INBIFO, D-5000 Köln 90

2501091336

Source: https://www.industrydocuments.ucsf.edu/docs/lghl0000

goat anti-rabbit IgG, no. 412o,
goat anti-rabbit IgG peroxidase-conjugated, no. 64oo,
goat anti-rabbit IgG alkaline phosphatase-conjugated, no. 65oo,
goat anti-rabbit IgG biotin-conjugated, no. 66oo,
goat anti-mouse IgG, no. 415o,
goat anti-mouse IgG peroxidase-conjugated, no. 645o,
goat anti-mouse IgG alkaline phosphatase-conjugated, no. 655o,
goat anti-mouse IgG biotin-conjugated, no. 665o,
avidin, no. BA-ooo,
biotin peroxidase-conjugated, no. BA-1o8,
biotin alkaline phosphatase-conjugated, no. BA-1o9
Medac,
D-2ooo Hamburg 36

Tween 2o (polyethylene sorbitan monolaurate), no. P 1379,
Sigma Chemie GmbH,
D-8o21 Taufkirchen

protein A 125I together with Bolton and Hunter reagent (1.1 GBq/mg), no. IM 112,
Amersham Buchler,
D-33oo Braunschweig

Procedure: according to Voller et al. (1979),

Alternative (A):

(1) coupling of immunogen to solid surface by incubating of 2oo ul of immunogen solution in PBS at 4 degrees centigrade overnight. Removal of the fluid, washing of the plates 3 times in PBS.

(2) incubation of the wells with PBS containing 1 o/o gelatin for 1 h at RT. Washing of the plates 4 to 6 times with PBS.

drying and storage of the plates in dry atmosphere until use

2501091337

Source: https://www.industrydocuments.ucsf.edu/docs/lghl0000

(3) addition of reference antiserum together with test sample and 1 o/o HSA, running of controls receiving known amounts of antigen instead of test sample, or non-immune serum or uncoated plates, for 2 to 24 h (to be determined). Washing of the plates 4 to 6 times with PBS

(4) addition of labeled 2nd antibody directed against rabbit gamma globulin (in case of rabbit antiserum used) or mouse gamma globulin (in case of mouse hybridoma antibodies used)

labeled second antibodies (alternatives):

(4.1) goat anti-rabbit or goat anti-mouse gamma globulin serum, labeled with 125I

(4.2) the same antisera labeled with 125I protein A

(4.3) the same antisera coupled with alkaline phosphatase

(4.4) the same antisera coupled with horse-radish peroxidase

(4.5) the same antisera coupled with biotin, added avidin, added enzyme coupled biotin (enzyme alkaline phosphatase or horse-radish peroxidase)

incubation for 2 to 24 h, washing 4 to 6 times in PBS

(5) for (4.1 and 4.2): cutting out of single wells and counting of radioactivity in a gamma-counter

Source: https://www.industrydocuments.ucsf.edu/docs/lghl0000

for (4.3 to 4.5): addition of the appropriate substrates and buffers for enzyme reaction, incubation for 3o min, reading of the color reaction visually or with photometer (peroxidase reaction at 492 nm, alkaline phosphatase at 4o5 nm)

Alternative (B): according to Engvall (198o),

(1) coupling of antibodies to solid surface. Incubation of 2oo ul of antiserum/well in microtiter plates overnight. Removal of the fluid, washing of the plates three times. Drying and storage of the plates in dry atmosphere until use

(2) incubation of the coated plates with labeled antigen, sample of unlabeled antigen, together with 1 o/o HSA, in PBS for 4 h. Removal of the fluid, washing of plates, running of controls each lacking one of the above steps or additives

(3) determination of radioactivity bound to plates

Scientific version: 8.Jan.81
Text version: 8.Oct.81

2501091339

Source: https://www.industrydocuments.ucsf.edu/docs/lghl0000

Radioimmunoassay determination of nicotine

Principle:	binding of antigen (nicotine) to specific antibody (anti-nicotine), one of them being attached to a solid substratum, the other one being labeled. Competition of the test sample antigen for the antibody binding sites, quantification of unknown nicotine concentrations by comparison with a standard curve obtained with known concentrations of nicotine
Time:	-
Sample material and quantity:	plasma, or test compound solution o.1 ml
Results expressed in:	ng/ml
Equipment:	liquid scintillation counter: Mark 3 model 68oo, W. Zinsser, D-6ooo Frankfurt/Main PVC microtiter plates, Cooke via Dynatech, D-731o Plochingen
Chemicals and reagents:	G-3H-nicotine d-bitartrate, no. TRA.428, Amersham Buchler, D-33oo Braunschweig or nicotine (pyrrolidine-2 14C), 1.48 to 2.22 GBq/mmol (4o to 6o mCi/mmol) dissolved in benzene, Mr 123.1, no. NEC-689, New England Nuclear, D-6o72 Dreieich

2501091340

(a) general background information on RIA: Skelly et al. (1973)

Source: https://www.industrydocuments.ucsf.edu/docs/lghl0000

anti-nicotine antiserum (rabbit, mouse), monoclonal anti-nicotine antibodies (mouse),
INBIFO,
D-5ooo Köln 9o

goat anti-rabbit IgG, no. 412o,
goat anti-rabbit IgG peroxidase-conjugated, no. 64oo,
goat anti-rabbit IgG alkaline phosphatase-conjugated, no. 65oo,
goat anti-rabbit IgG biotin-conjugated, no. 66oo,
goat anti-mouse IgG, no. 415o,
goat anti-mouse IgG peroxidase-conjugated, no. 645o,
goat anti-mouse IgG alkaline phosphatase-conjugated, no. 655o,
goat anti-mouse IgG biotin-conjugated, no. 665o,
avidin, no. BA-ooo,
biotin peroxidase-conjugated, no. BA-1o8,
biotin alkaline phosphatase-conjugated, no. BA-1o9
Medac,
D-2ooo Hamburg 36

Tween 2o (polyethylene sorbitan monolaurate), no. P 1379,
Sigma Chemie GmbH,
D-8o21 Taufkirchen

protein A 125I together with Bolton and Hunter reagent (1.1 GBq/mg), no. IM 112,
Amersham Buchler,
D-33oo Braunschweig

Procedure: according to Voller et al. (1979), see FIGURE . (2, Alternative A)

Alternative (A): (1) coupling of immunogen to solid surface by incubating of 2oo ul of immunogen in PBS at 4 degrees centigrade overnight. Removal of the fluid, washing of the plates 3 times in PBS

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Source: https://www.industrydocuments.ucsf.edu/docs/lghl0000

(2) incubation of the wells with PBS containing 1 o/o gelatin for 1 h at RT. Washing of the plates 4 to 6 times with PBS.

drying and storage of the plates in dry atmosphere until use

(3) addition of reference antiserum together with test sample and 1 o/o HSA, running of controls receiving known amounts of antigen instead of test sample, or non-immune serum or uncoated plates, for 2 to 24 h (to be determined). Washing of the plates 4 to 6 times with PBS

(4) addition of labeled second antibody directed against rabbit gamma globulin (in case of rabbit antiserum used) or mouse gamma globulin (in case of mouse hybridoma antibodies used)

labeled second antibodies (alternatives):

(4.1) goat anti-rabbit or goat anti-mouse gamma globulin serum, labeled with 125I

(4.2) the same antisera labeled with 125I protein A

(4.3) the same antisera coupled with alkaline phosphatase

(4.4) the same antisera coupled with horse-radish peroxidase

(4.5) the same antisera coupled with biotin, added avidin, added enzyme coupled biotin (enzyme alkaline phosphatase or horse-radish peroxidase)

incubation for 2 to 24 h, washing 4 to 6 times in PBS

(5) for (4.1 and 4.2): cutting out of single wells and counting of radioactivity in a gamma-counter

2501091342

Source: https://www.industrydocuments.ucsf.edu/docs/lghl0000

for (4.3 to 4.5): addition of the appropriate substrates and buffers for enzyme reaction, incubation for 3o min, reading of the color reaction visually or with photometer (peroxidase reaction at 492 nm, alkaline phosphatase at 4o5 nm)

Alternative (B): according to Engvall (198o),

(1) coupling of antibodies to solid surface. Incubation of 2oo ul of antiserum/well in microtiter plates overnight. Removal of the fluid, washing of the plates three times. Drying and storage of the plates in dry atmosphere until use

(2) incubation of the coated plates with labeled immunogen, sample of unlabeled immunogen, together with 1 o/o HSA, in PBS for 4 h. Removal of the fluid, washing of plates, running of controls each lacking one of the above steps or additives

(3) determination of radioactivity bound to plates

Scientific version: 9.Jan.81
Text version: 8.Oct.81

2501091343

Source: https://www.industrydocuments.ucsf.edu/docs/lghl0000

Determination of RNA

Principle:	photometric determination of the colored complex between orcinol and the sugar of RNA
Time	
Sampling:	-
Determination:	-
Sample material and quantity:	purified nucleic acid and fraction, approx. 1oo ul
Results expressed in:	ug
Equipment:	spectrophotometer: model 555, Perkin Elmer GmbH, D-777o Überlingen shaking water bath: no. 3147, Köttermann, D-3165 Hänigsen
Chemicals and reagents:	orcinol, no. o 1875, Sigma Chemie GmbH, D-8o21 Taufkirchen RNAse, yeast, no. 1o9126, Ribonucleic acid, no. 1o9215, Boehringer Mannheim GmbH, D-68oo Mannheim 31
Procedure:	according to Schneider, W. C., in: Colowick, S. P., Kaplan N.O. (Eds.): Methods in Enzymology, Vol. 3, New York-London: Acad. Press, 1957, pp. 68o-684
Scientific version:	17.Mar.8o
Text version:	28.Sep.81

2501091344

Source: https://www.industrydocuments.ucsf.edu/docs/lghl0000

SDS_polyacrylamide_gel_electrophoresis

Principle: separation of negatively charged complexes of proteins with a detergent (SDS) acccording to their relative molecular mass in an electrical field across a vertical polyacrylamide gel (1o o/o) with defined pore size and a discontinuous buffer system

Time

Sampling: on days ..

Determination: within ... days after sampling

Sample material and quantity: protein SDS complexes 5 to 3o ul equiv. to 5 to 3o ug protein/slot

Results expressed in: -

Equipment:

magnetic stirrer: Ika Combimag RCO,
Janke und Kunkel GmbH,
D-7813 Staufen

pH meter: PW 94o9,
glass electrode: CA/14o2,
Philips GmbH,
D-35oo Kassel

syringes: Hamilton, 1o or 5o ul,
Parafilm, via
Hirtz und Co.,
D-5ooo Köln 5o

glass tubes:
length: 12o mm
inner diameter: 4 mm

glass cell: Desaga Doppeltrennzelle,
thickness: 1.5 mm
width: 22o mm
length: 11o mm
volume: 37 ml

electrophoresis apparatus: system Havana,
Desaga GmbH,
D-69oo Heidelberg

power supply: no. 21o3,
LKB Instruments,
D-8o32 Gräfelfing

2501091345

Source: https://www.industrydocuments.ucsf.edu/docs/lghl0000

Chemicals and reagents:

acrylamide, no. A 8887,
N,N'-methylene-bis-acrylamide (Bis),
no. M 7256,
trizma base (Tris), no. T 15o3,
N,N,N',N'tetramethylethylenediamine
(TEMED), no. T 8133,
glycine, no. G 7126,
Sigma Chemie GmbH,
D-8o21 Taufkirchen

ammonium persulfate, no. 13375,
sodium dodecyl sulfate (SDS), no.
2o76o,
Serva Feinbiochemica GmbH,
D-69oo Heidelberg 1

sucrose, no. 7654,
E.Merck,
D-61oo Darmstadt 1

gel composition:

acrylamide	1o	o/o
Bis	o.15	o/o
ammonium persulfate	o.o25	o/o
TEMED	o.oo2	o/o
buffer	o.375	mol/l

electrode buffer composition:

Tris	25	mmol/l
glycine	o.2	mol/l
SDS	o.1	o/o
final pH (a)	8.3	

Procedure:

glass cells or tubes placed in buffer,
o.o25 mol Tris, o.192 mol glycine,
o.1 o/o SDS, pH 8.3 at 9 degrees cen-
tigrade

samples applied with Hamilton syringe
current: 94 mA for 2 glass cells or
1 mA/tube for cylindrical gels
starting voltage: approx. 9o V

tracking dye velocity: approx. 2 cm/h

Scientific version: 7.Jan.81
Text version: 25.Jun.81

(a) in absence of SDS

2501091346

Source: https://www.industrydocuments.ucsf.edu/docs/lghl0000

Serum preparation

Principle:	after clotting separation of serum from blood clot by centrifugation
Time	
Sampling:	-
Determination:	on day of sampling
Sample material and quantity:	blood, approx. 1.2 ml
Results expressed in:	-
Equipment:	centrifuge: type "Eppendorf", no. 5412, micro vials: type "Eppendorf", no. 381o, polypropylene, Netheler und Hinz, D-2ooo Hamburg 63
Chemicals and reagents:	-
Procedure:	blood samples kept in closed micro vials at RT for approx. 3o min.
Centrifugation:	9.8x1oE4 m/s2 (= 995o x g) for 2 min at RT. Transfer of serum supernatant to fresh micro vial
Storage:	at o to 4 degrees centigrade
Scientific version:	1.Sep.8o
Text version:	8.Oct.81

2501091347

Source: https://www.industrydocuments.ucsf.edu/docs/lghl0000

Determination_of_excised_skin_area

Principle:	planimetric determination of area of excised skin spread on graph paper
Time:	(a)
Sample material and quantity:	excised skin area, 1 piece/mouse
Results expressed in:	mm2
Equipment:	digitizer: MOP-AM o3, Kontron Messgeräte, D-8o57 Eching
Chemicals and reagents:	-
Procedure:	drawing of skin areal contours on graph paper planimetric determination of area with digitizer equipment (a)
Scientific version:	15.Apr.81
Text version:	25.Jun.81

(a) Details will be given in the report

2501091348

Source: https://www.industrydocuments.ucsf.edu/docs/lghl0000

Silanization of glass surfaces

Principle:	covalent binding of silane derivation to hydroxyl groups of glass surface in order to prevent electrostatic cell adherence
Time	
Sammpling:	-
Determination:	-
Sample material and quantity:	pasteur pipettes or other glass ware
Results expressed in:	-
Equipment:	-
Chemicals and reagents:	dichloro-dimethylsilane (DCDS), no. 803452, Merck-Schuchardt, D-8011 Hohenbrunn
Procedure:	immersion of glass in DCDS solution (1 ml/l DCDS in acetone), for 10 s, glass then rinsed 5 times with distilled water and dried in hot air (50 degrees centigrade) for 1 h
Scientific version:	22.Aug.79
Text version:	29.Sep.81

2501091349

Source: https://www.industrydocuments.ucsf.edu/docs/lghl0000

Determination of sodium concentration

Principle:	flame photometric determination of the intensity of the characteristic sodium emission in relation to the emission of an internal lithium standard
Time	
Sampling:	-
Determination:	within .. .. after sampling
Sample material and quantity:	(1) urine, 24 ul (2) perfusate supernatant, 24 ul (3) serum, 24 ul
Results expressed in:	mmol/l
Equipment:	KLiNa-flame photometer, no. 65271o, Beckman Instruments GmbH, D-8ooo München 4o
Chemicals and reagents:	internal lithium-standard: 15 mmol/l, no. 663229 sodium/potassium standard: sodium: 14o mmol/l, potassium: 5 mmol/l, no. 663223, Beckman Instruments GmbH, D-8ooo München 4o
Procedure:	according to manufacturer's recommendations
Scientific version:	6.Mar.79
Text version:	24.Sep.81

2501091350

Source: https://www.industrydocuments.ucsf.edu/docs/lghl0000

Staining of proteins in polyacrylamide gels with Coomassie blue

Principle:	binding of stain to fixed proteins, removal of excess dye by diffusion
Time:	fixation immediately after electrophoresis to prevent diffusion of protein
Sample material and quantity:	-
Results expressed in:	-
Equipment:	diffusion destainer for round gels: Bio-Rad Laboratories GmbH, D-8ooo München 5o diffusion destainer for flat gels: Desaga GmbH, D-69oo Heidelberg 1
Chemicals and reagents:	propanol-2, no. 9634, acetic acid, no. 62E, Coomassie brilliant blue R25o, no. 12553, E. Merck, D-61oo Darmstadt 1
Procedure:	fixation: at least 1 h in circulating fixation solution: 25o ml/l propanol-2, 1oo ml/l acetic acid staining: up to 1 h in fixation solution plus 125 g/l filtered Coomassie brilliant blue
Scientific version:	13.Oct.8o
Text version:	25.Jun.81

2501091351

Source: https://www.industrydocuments.ucsf.edu/docs/lghl0000

Storage of clones

Principle:	freezing of cells for unlimited preservation
Time:	-
Sample material and quantity:	1 to 5x1oE6 cells/(ml x tube)
Results expressed in:	-
Equipment:	cell freezer controller and liquid nitrogen refrigerator, Union Carbide, Durham, United Kingdom Nunc Kryo-tubes 2 ml, no. N 1o76-1/3 634o1, Nunc GmbH, D-62oo Wiesbaden
Chemical and reagents:	dimethyl sulfoxide (DMSO), no. 9678, E. Merck, D-61oo Darmstadt 1 fetal calf serum, no. 629, Difco Laboratories, Detroit, Michigan, USA
Procedure:	cells collected, centrifuged with 9.8x1oE3 m/s2 (= 1ooo x g) for 1o min and resuspended in a mixture containing 9oo ml/l fetal calf serum and 1oo ml/l DMSO, frozen down in programmed cell freezer and stored in liquid nitrogen
Scientific version:	2o.Nov.8o
Text version:	8.Oct.81

2501091352

Source: https://www.industrydocuments.ucsf.edu/docs/lghl0000

Surgical Preparation

Equipment:	cotton thread, 1oo o/o cotton, Amann 2ooo
	PVC tubing: inner diameter: 2 mm outer diameter: 3 mm with catheter tip
	PVC catheter, Braunüle type o, sterile and pyrogen-free, Braun Melsungen AG, D-35o8 Melsungen
	syringes: 5 ml, plastic, sterile and pyrogen-free, Pharma-Plast GmbH, D-33oo Braunschweig
Chemicals and reagents:	urethane administration solution: 233 g/l bidistilled water, urethane, no. 849o, E. Merck, D-61oo Darmstadt 1
	heparin administration solution: 1oo U/ml 9 g/l sodium chloride solution, heparin, no. 2459o, Serva Feinbiochemica GmbH und Co., D-69oo Heidelberg 1

Procedure

Anesthesia:	intraperitoneal administration of 1.18 g urethane/kg BW equivalent to 13.3 mmol/kg BW
Nerve and tendon preparation:	blunt preparation of the Nervus ischiadicus on each HL and loose clasping with cotton thread. Preparation of both Tendi m. tricipitis surae (Achilles), fixation of cotton thread in tendon, separation of tendons from Tuber calcanei
Blood vessel operation:	tight ligatures at the following blood vessels (venae and arteriae simultaneously) or organs in the given order (see also FIGURE .):

2501091353

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A. epigastrica inferior
A. circumflexa ilium profunda
A. iliaca interna
A. sacralis mediana
A. mesenterica inferior
A. mesenterica superior
Urinary bladder and prostate
A. renalis
A. testicularis
A. gastrica sinistra
A. hepatica
Aa. lumbales
A. inliaca lumbales

loose ligature around A. abdominalis and Vena cava inferior caudally to A. renalis

tight ligature around A. abdominalis and Vena cava inferior cervically to A. renalis (= start of circulation standstill)

insertion of PVC tube into incision of V. cava inferior, fixation of tube with caudal ligature

insertion of PVC catheter into incision of A. abdominalis, fixation of catheter with caudal ligature

Heparinization: instillation of approx. 2o ml heparin solution with syringe through PVC catheter into HL.

Killing: perforation of the diaphragm immediately after instillation of heparin solution

Isolation of HE from supper part of body: fixation of steel bar perpendicular to body axis at the lower kidney poles

Scientific version: 3o.Jan.8o
Text version: 19.Aug.81

2501091354

Source: https://www.industrydocuments.ucsf.edu/docs/lghl0000

Determination of temperature

Principle:	resistance thermometer (Pt 1oo) reading
Time:	.. min after start of perfusion
Sample material and quantity:	perfusate, sites of determination: (see FIGURE ..) (1) reservoir (2) venous tubing
Results expressed in:	degrees centigrade
Equipment:	digital thermoelement: R4o/2, Messgeräte-Werk Lauda, Dr. R. Wobser KG, D-697o Lauda-Königshofen digital thermometer: H11, Hugo Sachs Elektronik KG, D-78o1 March-Hugstetten/Freiburg
Chemicals and reagents:	-
Procedure:	platinum probe (Pt 1oo) completely submersed in perfusate stream directly behind the perfused organ and in reservoir
Scientific version: Text version:	3.Dec.79 7.Oct.81

250109135

Source: https://www.industrydocuments.ucsf.edu/docs/lghl0000

Thin-layer chromatography

Principle:	chromatographic separation based on differential adsorption to and/or distribution of substances in a mobile phase and stationary cellulose thin-layer phase
Time:	within 2 h after preparation of test or reference substance solution
Sample material and quantity:	test or reference substances dissolved in bidistilled water, 1 to 1o ul equiv. to 1o to 1oo ug/plate
Results expressed in:	Rf (a)

Equipment

Chromatography:	thin-layer plates: cellulose F layer on glass plate, no. 57o8, E. Merck, D-61oo Darmstadt 1 spray gun: C. Desaga GmbH, D-69oo Heidelberg filter paper: no. 2524o, UV-lamp, no. 292oo, Camag, D-1ooo Berlin
Photography:	MP4 Land camera, Land Polacolor 2 film, type 668, Polaroid GmbH, D-6ooo Frankfurt/Main-Niederrad 1
Other equipment:	analytical balance: model 2oo1 MP2, Sartorius GmbH, D-34oo Göttingen

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(a) quotient of test or reference substance migration from point of application and distance of mobile phase front migration

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Chemicals and reagents:

bromophenol blue, no. 8122,
butanol, no. 199o,
methanol, no. 6oo7,
ammonia, no. 5426,
formic acid, no. 264,
acetic acid, no. 62
dioxane, no. 9671,
E. Merck,
D-61oo Darmstadt 1

benzol, no. 8o14,
chloroform, no. 7152,
Baker Chemikalien,
D-6o8o Gross-Gerau

ethanol, denaturated,
Bundesmonopolverwaltung für Branntwein,
D-5ooo Köln 1

Procedure

Thin-layer chromatography: according to Stahl: Dünnschicht-Chromatographie, Berlin, Heidelberg: Springer Verlag (1967), S. 293

mobile phase (solvent phase mixtures: see TABLE .)

Photography: reflected white light

Scientific version: 24.Oct.8o
Text version: 19.Jul.81

2501091357

Source: https://www.industrydocuments.ucsf.edu/docs/lghl0000

Preparation of tissue homogenate supernatant = S9 fraction

Principle:	mechanical grinding and centrifugation with 8.8x1oE4 m/s2 (= 9ooo x g) of tissue
Time	
Sampling:	on day ...
Determination:	within ... days after sampling
Sample material and quantity:	organs, ... pooled/group
Results expressed in:	-
Equipment:	homogenizer: Potter-Elvehjem, glass, with motor-driven teflon pestle, Braun Melsungen AG, D-35o8 Melsungen Multifix, model M 8o, Alfred Schwinher, D-7o7o Schwäbisch-Gmünd centrifuge: RC-5B, rotor: SS-34, Du Pont Instruments, D-635o Bad Nauheim freezer: no. 8218, Forma Scientific, Marietta, Ohio, USA tubes: polycarbonate, no. 3137, polypropylene, no. 3138, Du Pont Instruments, D-635o Bad Nauheim polypropylene, no. 381o, Netheler und Hinz, D-2ooo Hamburg 63
Chemicals and reagents:	potassium chloride, no. 4936, E. Merck, D-61oo Darmstadt 1

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Source: https://www.industrydocuments.ucsf.edu/docs/lghl0000

Procedure	according to Ames B.N., McCann, J. and Yamasaki, E., Mutation Research 31: 347-364 (1975)
Killing:	stretching of spine, followed by 3o s exsanguination from inferior vena cava and abdominal aorta
	short submersion of organs in 15o mmol/l potassium chloride, pH 7.o, determination of wet weight, freezing of whole organ in liquid nitrogen
Storage:	in jars at minus 8o degrees centigrade
Homogenization:	after addition of 3 volumes of 15o mmol/l potassium chloride, pH 7.o, to the organ wet weight (1 g equiv. to 1 ml) mechanical grinding in Potter-Elvehjem apparatus with motor-driven teflon pestle, approx. 2oo rpm, 4 degrees centigrade
	glassware and equipment in contact with homogenate: precooled
Centrifugation:	8.8x1oE4 m/s2 (= 9ooo x g), for 1o min, 4 degrees centigrade
Storage of S9 fraction:	minus 8o degrees centigrade, 1 ml aliquots

Scientific version: 7.Dec.8o
Text version: 12.Jul.81

2501091359

Source: https://www.industrydocuments.ucsf.edu/docs/lghl0000

Determination of triglyceride concentration

Principle:	photometric determination of the NADH oxidation during the formation of lactate from pyruvate, which is produced from phosphoenolpyruvate by pyruvate kinase (EC 2.7.1.4o). ADP necessary for this reaction is formed from ATP and glycerol (glycerokinase EC 2.7.1.3o). Glycerol is obtained from triglycerides after hydrolysis by lipase (EC 3.1.1.3) and esterase (EC 3.1.1.1)
Time	
Sampling:	on day ..
Determination:	on day of sampling
Sample material und quantity:	serum, 5 ul
Results expressed in:	mmol/l
Equipment:	ABA-1oo Bichromatic Analyzer, Abbott Laboratories, Diagnostic Division, So. Pasadena, California 91o3o, USA
Chemicals and reagents:	test set Triglycerides, UV method, no. 126 o12, Boehringer Mannheim GmbH, D-68oo Mannheim 31
Procedure:	according to Wahlefeld, A.W. in: Bergmeyer, H.U. (Ed.): Methoden der enzymatischen Analyse, 3rd ed., Vol. 2, Verlag Chemie Weinheim, 1974, p. 1878
	photometric determination:
	wavelength 1: 34o nm
	wavelength 2: 38o nm
Scientific version:	1.Sep.78
Text version:	28.Sep.81

2501091360

Source: https://www.industrydocuments.ucsf.edu/docs/lghl0000

Determination of urea concentration

Principle:	photometric determination of NADH oxidation during the reaction of alpha ketoglutarate with ammonia (glutamate dehydrogenase, EC 1.4.1.2), formed from serum urea by urease (EC 3.5.1.5)
Time	
Sampling:	-
Determination:	-
Sample material and quantity:	serum, 2.5 ul
Results expressed in:	mmol/l
Equipment:	ABA-1oo Bichromatic Analzyer, Abbott Laboratories, Diagnostics Division, So. Pasadena, California 91o3o, USA
Chemicals and reagents:	test set BUN A. Gent, no. 6oo7, Abbott Laboratories, Diagnostics Division, So. Pasadena, California 91o3o, USA
Procedure:	according to Talke, H., Schubert, G.E., Klin. Wochenschr. 43: 174 (1965), with modifications by Abbott Diagn. photometric determination: wavelength 1: 38o nm wavelength 2: 34o nm
Scientific version:	1.Sep.78
Text version:	23.Sep.81

2501091361

Source: https://www.industrydocuments.ucsf.edu/docs/lghl0000

Determination of wet weight

Principle:	gravimetric determination
Time	
Sampling:	on day ..
Determination:	immediately after dissection
Sample material and quantity:	rat organs, 5 organs pooled
Results expressed in:	g
Equipment:	scales: no. 1212 MP, Sartorius GmbH, D-34oo Göttingen
Chemicals and reagents:	-
Procedure:	-
Scientific version:	21.Sep.8o
Text version:	9.Oct.81

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Source: https://www.industrydocuments.ucsf.edu/docs/lghl0000